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Discussion

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Discussion

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Discussion

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Discussion

c) further studies in nephrotic patients - - Robert Slater

Discussion

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Foreword

The purpose of the annual conference on the Nephrotic Syndrome is to provide an informal exchange of ideas and comments with preliminary reports on research or progress by workers in this field The 5th annual conference notably achieved these aims. The success of the meetings owes much to the excellent program arranged by the hosts, Drs Milton Rapoport and Wallace McCrory and to the excellent cooperation of the participants We are grateful to the National Nephrosis Foundation for underwriting these conferences and making possible the publication of their proceedings.

Boston
February, 1954

Jack Metcoff

I. Studies of Normal Glomerular Structure

CHAIRMAN RAPOPORT. For those that have been here before you know that this meeting is rather informal That is, there are no formal papers read as such. There are presentations with, we hope, as free and active and vigorous discussions as the material will provoke I think the wisest thing is to get right into the program, and the first material is to be presented by Dr. C. Vincent Hall.

Now, Dr Hall, if you will please take over.

DR. HALL Dr Rapoport and conference members. It is with considerable temerity that we appear before a group of men experienced in a field in which we can claim no experience Only by Dr. Metcoff's assurances that this group meets very informally and welcomes the presentation of tentative ideas and preliminary accounts of research in progress such as ours, were we encouraged to come here today. The factual basis of our talk this afternoon has been gained largely through the capable technical assistance of two workers in the Division of Biological and Medical Research at the Argonne National Laboratory, whose names should be associated with the work, Evans Roth, electron microscopist, and Mrs. Vera Johnson, technician. (1)

Our approach in applying the technics of electron microscopy to the problem of the structure of the glomerulus, was begun with the perhaps overly ambitious hope of relating its structure to function, a goal which remains elusive, but the results to date have been sufficiently promising to encourage our continued interest and effort.

First a brief statement about the technic This study has been based chiefly on kidneys of healthy, adult male rats, whose tissues were fixed largely by perfusion of nembutal anesthetized animals with varied fixatives, all buffered usually with phosphate buffers to about pH 7 2 Three fixatives were used, 1) 5% formalin in physiological salt solution 2) formalin-alcohol (1 pt formalin + 2 pts 80% ethyl alcohol), 3) and 1% OsO_4 solution Shortly after perfusion with the fixative, thin slices were cut from the kidneys and immersed in fresh buffered 1% OsO_4. Some rat and the human tissue was fixed only by immersion of thin slices cut from kidneys directly after their removal. The human kidney was made available by the kind cooperation of Dr. J. B Christie of Champaign, Illinois It was a hydronephrotic kidney, removed surgically, with some areas of apparently normal cortex, from which our tissue was taken.

After dehydration, the tissues were embedded in a methacrylate mixture and sectioned at 0 1 or 0.5 u with a glass knife in a Minot International Microtome. They were mounted on grids on collodion membranes, and examined in an R.C.A. EMU microscope, after dissolving the plastic out of the tissue by placing the grids for 30 minutes in toluene

This study has also employed 2 u serial sections prepared for the light microscope in a rather similar fashion, except they were stained by Van Gieson's procedure

(1) Hall, B V , Roth, Evans, and Johnson, Vera. 1953. "The Ultramicroscopic Structure and Minute Functional Anatomy of the Glomerulus," Federation Proceedings, 12: 467-468

to demonstrate connective tissue. We were fortunate in obtaining a number of serial sections with the electron microscope which have been most valuable in developing a three dimensional concept from one-tenth and one-twentieth u sections.

As Mollendorf(2) emphasized years ago, it is extremely important in studying the glomerulus to use specifically stained, thin *sections of tissues, preferably fixed by* perfusion. Fig. 1* shows how much more can be seen in such a section than in a standard 6 to 10 u section. Note the well-defined basement membrane outlining the capillaries and Bowman's capsule. Striations and processes in the cytoplasm on the surface of the capillaries are clearly visible, and at the top center a dark round cytoplasmic body, which is a Golgi body of the Hirsch-Baker type. The complicated capillary *pattern gives the impression of numerous individual capillaries attached together by* the basement membrane. A darkly staining tissue, and some nuclei lying between the parallel sections of the basement membrane may also be seen in these so-called connections between the apparently separate capillaries. To understand the glomerular capillaries well, they must be studied in whole freshly dissected glomeruli or in intact cleared injected ones, as Bowman(3) and Vimtrup(4) among others have done. When *this is done, it is soon discovered that the appearance of numerous separate capillaries* seen in section must result from the sectioning knife cutting through folds, sacculations and bends of a relatively few large capillaries. For example, in this section there can be counted on the right side about 26 separate apparent capillaries, some only a few u in diameter Yet, when the serial sections are followed on the right side of this particular section, it is found that there are in reality not more than 4 capillaries in this region

The misimpression that each opening in a sectioned glomerulus represents a separate capillary and misimpressions about facts of the development of a glomerulus have led to several erroneous concepts about glomerular structure which are not supported by the evidence of the electron micrographs, the two mu serial sections, or the facts of development as stated by Huber(5).

Following Zimmerman's(6) conception of the glomerulus, a section of a glomerulus as pictured in Fig. 2 must be interpreted as representing (on the left) a group of

(2) v Möllendorff, Wm. 1927-1928 "Einige Beobachtungen über den Aufbau des Nierenglomerulus," Zeit. für Zellforsch. u Mikro. Anat., 6·441-450.

(3) Bowman, W 1842. "On the Structure and Use of the Malpighian Bodies of the Kidney, with Observations on Circulation through that Gland," Phil. Trans. of the Roy. Soc. of London, 132:57-80

(4) Vimtrup, Bj. 1928. "Number, Shape, Structure, and Surface Area of Glomeruli in Man and Animals," Am Jour Anat , 41 123-151.

(5) Huber G. Carl 1909-10. "The Morphology and Structures of the Mammalian Renal Tubule." The Harvey Lectures, pp. 100-149

(6) Zimmermann, K. W. 1933. "Über den Bau des Glomerulus der Saügerniere," Zeitschrift f. Mikro Anat. Forsch , 32 176-278

*Explanation of Figures, legends for figures. All figures have a line on them representing one micron at the magnification of the figure. All figures except Figs. 13, 14, and 19 are of rat tissues





barely visible on lower left Within the basement membrane all nuclei cells and cell products are endothelial and all space intracapillary Fixation etc as in Fig 1 X4 000

geneous material, covered externally everywhere by an intricate maze of minute processes, some just above, some just below the limits of the resolving power of the light microscope. The luminal surface of this thin, dense homogeneous layer, in some areas is covered by a thinner delicate net-like layer of highly specialized cortical endothelial cytoplasm (ectoplasm), which cannot be resolved by the light microscope, in other areas by thicker layers and larger masses of the inner or medullary portion of the entothelial cytoplasm (endoplasm). We shall use the term lamina densa for the discrete smooth, homogeneous continuous dense membrane visualized in the glomerular capillary wall by the electron microscope. For the thicker complex of structures, which includes the lamina densa and the minute processes on its external surface and the net-like lining layer on its luminal surface, when visualized as one structure by the light microscope, we continue to use the term basement membrane.

Although the two nuclei of Fig. 3 appear very much alike, one must be designated as an interstitial cell by employing Zimmerman's concept, because it is apparently separated completely from the lumen of the capillary; and the other, by virtue of the fact that its nucleus at least partly projects into the capillary lumen, by definition must be identified as an endothelial cell. The rather dark cytoplasm between the apparently separate capillaries, by definition again, would be termed mesangial connective tissue, which has been described by supporters of this concept as consisting largely of collagen fibers. Brief examination of this figure and others to follow leaves no doubt that the material which lies around these nuclei is not fibrous connective tissue. It is actually endothelial cytoplasm. The evidence for such a statement is two-fold. Electron micrographs of thin well-fixed sections such as this figure and others as Fig. 8 not only show that there are no collagen fibers in this tissue, but they at the same time show cytoplasm with numerous small mitochondria, which in some sections are characterized by internal structure similar to that described by Palade (13). Examination of Oberling, Gautier and Bernhard's(14) reproductions of electron micrographs of comparable regions connecting openings labeled as capillaries, reveals only dense black areas completely lacking detail, which they interpret as mesangium. No doubt their sections were too thick and the fixation inadequate to permit them to observe the cytoplasmic nature of these areas. It should be pointed out that the recent studies of Pease and Baker(15), Dalton(16) and Rinehart et al(17) using the technics of both electron microscopy and histochemistry fail completely to support the mesangial concept.

(13) Palade, George E. 1952. "The Fine Structure of Mitochondria," Anat. Rec., 114:427-451.
(14) Oberling, Charles, Gautier, A., and Bernhard, W 1951. "La Structure des Capillaires Glomérulaires vue au Microscope Électronique," La Presse Medicale, 4 Juillet, 1951, 59 - No. 45 938-940.
(15) Pease, Daniel C., and Baker, R.F. 1950. "Electron Microscopy of the Kidney," Am Jour. of Anat., 87:349-390
(16) Dalton, A.J. 1951. "Structural Details of Some of the Epithelial Cell Types in the Mouse as Revealed by the Electron Microscope," Jour. Natl. Cancer Inst., 11:1163-1185.
(17) Rinehart, James F, Farquhar, M.F., Jung, H.C., and Abul-Haj, S.K. 1953. "The Normal Glomerulus and Its Basic Reactions in Disease," Am. J. of Pathology, 29:21-32.

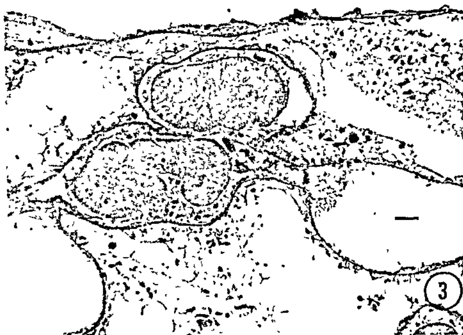




Fig 4 Photomicrograph of glomerulus showing folded capillary wall All nuclei within the basement membrane are endothelial podocyte nucleus with large Golgi body on lower left other Golgi bodies at top left and left of center On the left the thick heavy basement membrane of the capsule contrasts with the much thinner capillary basement membrane Technic as in Fig 2 X3 200

Fig 3 Electron micrograph of glomerular capillary wall showing two endothelial nuclei one of which is fully, the other partially surrounded by endothelial cytoplasm containing mitochondria Characteristically less dense cytoplasm of podocyte is shown in lower center forming trabeculae and pedicels Capillary lumen at top lower left and on right with micron marker Buffered OsO_4 fixative X6 000

geneous material, covered externally everywhere by an intricate maze of minute processes, some just above, some just below the limits of the resolving power of the light microscope. The luminal surface of this thin, dense homogeneous layer, in some areas is covered by a thinner delicate net-like layer of highly specialized cortical endothelial cytoplasm (ectoplasm), which cannot be resolved by the light microscope, in other areas by thicker layers and larger masses of the inner or medullary portion of the entothelial cytoplasm (endoplasm). We shall use the term lamina densa for the discrete smooth, homogeneous continuous dense membrane visualized in the glomerular capillary wall by the electron microscope. For the thicker complex of structures, which includes the lamina densa and the minute processes on its external surface and the net-like lining layer on its luminal surface, when visualized as one structure by the light microscope, we continue to use the term basement membrane.

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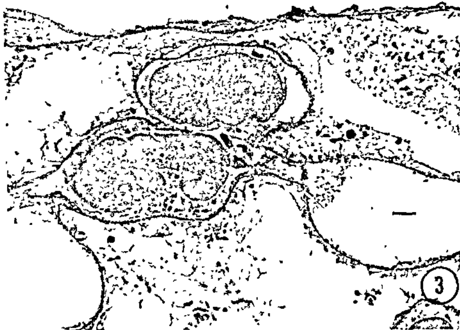




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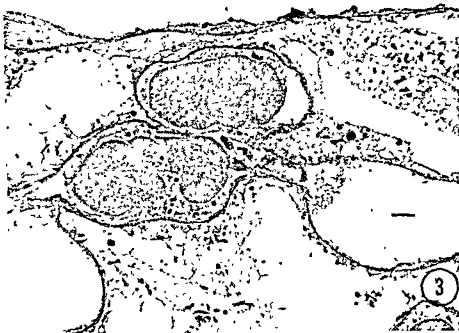




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Inspection of Fig. 4 makes it clear that the two cell types in the glomerulus, the endothelial cells and the covering or so-called visceral epithelial cells of Bowman's capsule, which have been termed Deckzellen by Mollendorf(2), pericytes by Bargman (18), epicytes by Clara(19), lie on either side of what appears as a dark homogenous membrane, the basement membrane, which in stained sections appears as a dark line On the left one sees a much thicker structure which is the basement membrane of Bowman's capsule. Lying between the basement membranes of Bowman's capsule and the capillary are two cells, one elongated, a capsular cell, and the other with a very irregular nucleus and a large Golgi body of the Hirsch-Baker type. Such a Golgi body or a more complicated one, as can be seen at the top left edge of this figure, may be identified in association with the nucleus of each of the cells covering the glomerular capillaries.

Fig. 5 shows in an electron micrograph the two types of cells which comprise the glomerulus, two so-called epithelial cells on the left and three nuclei of endothelial cells on the right, with a small tip of a fourth endothelial nucleus in the center. This figure shows the characteristic irregularities of the nuclear membrane of the so-called epithelial cells, which we have termed podocytes. We use this term, not merely to increase the confusion of an already varied terminology, but because electron microscopy has made it clear that these cells differ extraordinarily from epithelia in general Each one of these cells gives rise to thousands of minute sub-microscopic processes which we have called foot processes or pedicels which differentiate these cells from all other known cells. The electron microscope has also shown that each podocyte is a highly specialized cell, seemingly maintaining itself separate from neighboring cells without the intervention of intercellular material.

The pedicels are the terminal or primary processes which appear here at the low magnification of this figure as separate external projections, forming a part of what would be recognized in the light microscope as the basement membrane of the capillary Pease and Baker in first describing these projections, described them incorrectly as being sections of elongated ridges in the basement membrane itself, but Dalton correctly interpreted them as minute cytoplasmic processes from the cells of the "visceral" layer of Bowman's capsule. In addition to the minute pedicels, this figure shows that the podocytes give rise to larger processes, which appear here about one mu wide from which the pedicels arise as lateral processes. We have called these second order processes "trabeculae." Before leaving this figure it should be emphasized that although only the nucleus in the upper right corner would be considered endothelial by supporters of the mesangial theory, the two nuclei lying near it are enclosed within the lamina densa of the basement membrane and are identical in appearance with the nucleus at the top right. We have no doubt that these three cells are all endothelial and the fact that two of them do not project into the capillary lumen is merely due to the fortuitous circumstance that the sectioning knife cut them both close to their intimate connection to the capillary wall. In other words, the edge of the capillary wall is sectioned here tangentially in a region where 4 endothelial nuclei are

(18) Bargman, W 1933. "Weitere Histologische Untersuchungen am Nierenkorperchen." Zeitscht. f Zellforsch. u. Mikro. Anat, 18 166-191

(19) Clara, Max. 1936 "Vergleichende Histobiologie des Nierenglömerulus und der Lungenalveole," Zeitscht f. Mikr Anat. Forschung, 40.147-280.

onclude that such apparent arches are the result of the
bliquely and tangentially to the long axis of the pedicels,
iving origin to two or more pedicels on the same side of
view. Numerous sections like the one pictured in Fig. 8
between two neighboring pedicels which take their origin
or less parallel to the trabecula giving rise to the first
in Fig. 9 which will be discussed later. The pedicels are
and from 1000-2000A at their greatest width, their base,
ensa They are about 1000-1500Å units in height and appear
they are sectioned perpendicularly. Some small seemingly
the external surface of the lamina densa are the nearest to
ions of pedicels in Fig. 8. A view of a pedicel in which it
0.2 u must be an oblique or tangential section. It may be
appear separated by a space of several hundred Angstrom
nsa. This space is often only 100A or less. However, in well-
cels and trabeculae in perpendicular section appear quite dis-
nsa, although they may be in close contact with it. We find that
the lamina densa is everywhere smooth. In no case has a section
be useful in affording a clear, detailed picture of the relationship
nsa and the pedicels and trabeculae covering it) given the slight-
ere are extensions of the lamina densa alternating with extensions
lls, as Oberling, Gautier and Bernhard believed they saw in their
sections.

nentioned in passing that Fig 8 shows clearly the nature of the
(endoplasm) with small mitochondria characteristically occurring
nuclei and occupying the areas termed interstitial, mesangial and
various light microscopists. This endothelial cell with its dense
e termed an interstitial cell by some observers, but note in the lower
he figure that the cytoplasm of this cell lines the capillary lumen and
ly lacks collagen fibers, which have been described as an important
e so-called mesangial or interstitial tissue. The intimate relationship
ina densa and the endothelial cell cytoplasm is clearly shown also This
l the frequent close proximity, seemingly direct contact, of endothelial
lamina densa suggest the endothelial origin of the lamina densa. This
also supported by the electron density of the endothelial cytoplasm which
ays close to the density of the lamina densa material On the other hand,
cytoplasm usually appears distinctly different and separate from lamina
al

s an electron micrograph showing pedicels and trabeculae cut tangentially.
gitation of the pedicels, as described by Pease and Baker and their origin
r processes, the trabeculae, is apparent. It is possible that regions like
e the trabeculae have narrowed to widths comparable to the widths of the
are characteristic of areas of the capillary wall where one podocyte ap-
and interdigitates with another. The independence of the individual pedicels
ent and only rarely do they seem to be so close as to touch a neighboring
The regions of apparent contact are so infrequent and so limited in extent that
ay be purely fortuitous. Between the pedicels and at their rounded and often





Fig 9 Electron micrograph showing tangential view of interdigitating pedicels leaving a small free area of lamina densa between them in the series forming the diagonal to the right of center In the group to the left of this the pedicels were cut higher up from their base so the lamina densa does not show only space which actually would be capsular space Capillary lumen at top and left Buffered 5% formalin fixation X24 000

Fig 10 Electron micrograph with lamina densa traversing across the top showing obliquely sectioned (the long ones) and [illegible]

X21 000

somewhat enlarged ends, one sees either space, or the surface of the lamina densa. There is at times a suggestion of a fine porosity in such tangential views of lamina densa, which will be seen more clearly in following sections. The space on the left and top of this section is capillary lumen and the specialized endothelial cytoplasm, comprising the lamina fenestrata with its large openings (around 1000Å), may be seen bordering these spaces.

Fig. 10 is presented to show the transition of undifferentiated endothelial cytoplasm into the specialized thin layer, the lamina *fenestrata, which appears somewhat* like a fisherman's net when viewed tangentially. Across the top of the figure may be seen the lamina densa with pedicels in close contact with its external surface. The *pedicels are obviously separate from and differ in electron density from the material* of the lamina densa. That the pedicels are discrete processes and that they are separated from each other so as to leave narrow strips of lamina densa exposed directly to the space of Bowman's capsule seems apparent in this and similar sections. The pedicels in this section along the top of the slide have been cut at varying angles, some the shortest, were cut perpendicularly or nearly so, and the longest, including all of those in the lower right corner, have been cut obliquely or tangentially.

It should be stated at this point that no suggestion of two basement membranes, whose existence has been implied by Zimmerman(6) and the supporters of the mesangial theory, and even by Allen(11), has appeared in the electron micrographs or the *photomicrographs of our material. At times longitudinal sections of the pedicels or* trabeculae may be made, and these may appear as a second membrane in electron micrographs, but they are never more than a mu or so long, and are never continuous. On the other hand, the lamina densa has always appeared continuous in sections free of artifacts. In poorly fixed sections the pedicels often appear to have spread laterally over the lamina densa, and they may then, especially at low magnifications, give the appearance of a second membrane which is usually limited and interrupted. Such an apparent second membrane we believe is an artifact.

Before leaving this section it should be mentioned that the obvious gaps between two endothelial cells projecting into the lumen have been followed in some sections *until the cells meet. It is possible to detect the two cell membranes in close contact,* but remaining separate, so we cannot say that *the endothelial cells form a complete* syncytium, although boundaries between cells have not been seen in the lamina fenestrata. *The endothelial cells closely join each other to form* a continuous lining and are not separate isolated cells as suggested by Bell(10) and others. The fact that intercellular boundaries do not appear with silver nitrate staining as shown by Bensley and and Bensley(20) must be related to the absence *of any or sufficient intercellular* material to stain so that the junction can be resolved by the light microscope.

It should also be mentioned here that quite contrary to *the statements of Bell(21)* and Mollendorf(2) the podocytes, epithelial or covering cells, do not greatly outnumber

(20) Bensley, R.R., and Bensley, R.D. 1930. "The Structure of the *Renal Corpuscle*," Anat. Rec., 47:147-176.
(21) Bell, E.T. 1936 "The Early Stages of Glomerulonephritis," Amer. J. Path., 12:801-825.





Fig 9 Electron micrograph showing tangential view of interdigitating [illegible]
densa betw[illegible]
the right [illegible]
pedicels we[illegible]
densa does [illegible]
capsular sp[illegible]
5% formalin [illegible] [illegible] 000

Fig 10 Electron micrograph with lamina densa traversing across the top [illegible]
and nearl[illegible]
pedicels o[illegible]
surface in[illegible]
lamina fen[illegible]
and undiff[illegible]
filled larg[illegible]
X21 000

the endothelial cells. Mollendorf gives a ratio of 10 Deckzellen to 1 endothelial cell. Careful counts of cell types in the 2 u serial sections followed from section to section, and of the cells in nearly a dozen large photomontages of electron micrographs, leaves no doubt that endothelial cells in the rat glomerulus outnumber the podocytes by 3 or 4 to 1. It may be pointed out that the large rather rounded openings in the undifferentiated cytoplasm of the endothelial cell on the left are not uncommonly observed in these cells. They are generally more or less filled by a plug of cytoplasm. It is difficult to attach either anatomic or physiologic significance to this arrangement.

Fig 11 is a typical tangential view of the specialization in the glomerular capillaries of the thin layer of cortical endothelial cytoplasm to form the lining network or lamina fenestrata. Since we have observed this structure frequently in both human and rat glomeruli fixed with three different fixatives, we believe it is a constant feature of a part of the glomerular capillary wall. The size and shape of the openings in the membrane is rather uniform, being irregular ovals varying usually from 600-1600Å in diameter The material of the mesh-work forms strands which in tangential view appear to be about 300-500Å wide while the nodes appear somewhat larger. In good sections cut perpendicular to the lamina fenestrata and lamina densa, the material of the mesh appears to be about 200-300Å thick with the nodes again appearing thicker and more dense At the nodes, short radial processes apparently composed of the same material that forms the two laminae, seem to support the lamina fenestrata away from the lamina densa In oblique sections, connections of the lamina fenestrata with the lamina densa at the nodes may appear as a continuous extension with no change apparent in the electron density.

The openings of the lamina fenestrata occur with a frequency of about 5×10^7 per cm . Since they are about 1000Å in diameter, about half the lamina fenestrata consists of open space Since the lamina fenestrata does not line the glomerular capillaries completely, as considerable areas of the capillary wall are covered by the more dense medullary (inner) endothelial cytoplasm and nuclei, perhaps, as an approximation, not more than 25 per cent of the lining of the glomerular capillaries affords free access to the lamina densa Chambers and Zweifach (22) have proposed the theory that the pores of the inter-endothelial cement are the structural basis underlying capillary permeability. Since there seems to be no evidence for inter-endothelial cement in the glomerular capillaries it does not seem possible to associate the permeability of the glomerular capillaries with it. It appears that the lamina fenestrata as described must be an important structural feature distinguishing glomerular capillaries, as far as is now known, from all other capillaries In view of the nearly total abscence of evidence for inter-endothelial cement in glomerular capillaries, we suggest that the large pores of the lamina fenestrata are basic structural features of glomerular capillaries with an important role in making it possible for the characteristically rapid glomerular filtration to occur independently of differentially conditioning molecular forces which determine diffusion rates. Direct experimental methods developed by Wearn and Richards (23) and others, and less direct but equally effective methods as developed by Smith (24)

(22) Chambers, Robert and Zweifach, B W 1947 "Intercellular Cement and Capillary Permeability." Physiological Reviews, 27 436-463.
(23) Wearn, J T and Richards, A N 1924-25 "Observations on the Composition of Glomerular Urine with Particular Reference to the Problem of Resorption in the Renal Tubule Amer J Physiol . 71 209-227.

and others have amassed a wealth of physiological and chemical data all of which establishes the unqualified conclusion that glomerular filtration occurs as a result of the hydrostatic pressure within the glomerular capillaries being sufficiently greater than the osmotic pressure of the non-filterable solutes (proteins) to force water with filterable solutes through the capillary wall, as an ultrafiltrate is forced through an ultrafilter. The mass of this quantitative data also establishes the fact that the concentration of individual solutes in the glomerular filtrate is not influenced by their respective diffusion rates, but is equal to, or nearly equal to their individual concentrations in the plasma. These facts are fully in keeping with the concept that glomerular filtration takes place through a system of preformed passages (pores) in the glomerular wall which serve as an ultrafilter in the same sense as a thin collodion membrane may serve as an ultrafilter. The best available evidence concerning the minute structure of the glomerular capillary wall, in no way contradicts, and as far as it is valid, completely supports the conclusion that in certain regions of the glomerular capillary wall there exists a functioning system of minute, simple and complex pores and channels which is the structural requisite of an ultrafilter.

Before leaving Fig. 11, it should be stated that Oberling, Gautier and Bernhard were the first to visualize the network lining the glomerular capillaries but they seem to have misinterpreted its true relationships and failed to recognize its possible significance. They describe, without presenting adequate photographic evidence to support their view, the lamina fenestrata (our term) as a honeycomb-trellis which functions to support a thin, endothelial pellicle, forming an intact, continuous lining of the capillary wall. We judge that their error results in part from their failure to find good perpendicular sections of the lamina fenestrata and thus their interpretation was based solely on none-too-clear, oblique and tangential sections of this thin fenestrated lining layer.

It should also be mentioned that Rinehart, et al (17) also pictured the endothelial lining network. We agree with their concept that the primary layer of the basement membrane is formed as a product of endothelial cytoplasm, but their interpretation of the origin of the lamina fenestrata has no support from our electron micrographs. Their suggestion that the lamina fenestrata material is extruded through the lamina densa by extension of the frond-like processes (pedicels) of the epithelial cells is based on their interpretation of stained sections viewed in the light microscope. It is impossible for the light microscope to resolve this mesh-work. Their interpretation of the lamina fenestrata is supported neither by their own published electron micrograph nor by any of hundreds acquired in this study However, our experience is in agreement with theirs in that collagen fibers were not found in the glomeruli.

In Fig. 11 a dark gray material may be seen through the openings of the lamina fenestrata which must be a thin tangential section of the lamina densa. In this section and in Fig. 12, and in similar sections, there may be seen an apparent fine porosity in such thin tangential sections, which appears to be unique to the lamina densa and the closely associated *lamina fenestrata substance* Our material includes many similar sections from several rats fixed with the three different fixatives. These rounded and oval minute openings may be seen through the large openings between the number

(24) Smith, Homer W. 1951. The Kidney: Structure and Function in Health and Disease. 1049 pp. New York: Oxford University Press.



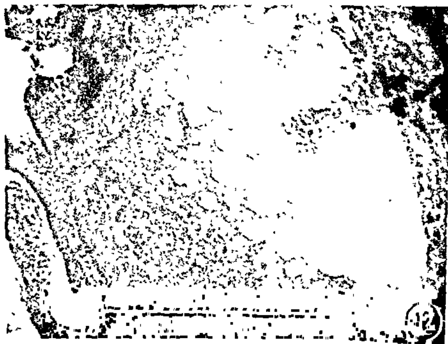


Fig 11 Electron micrograph showing lamina fenestrata in tangential view with thin section of lamina densa visible through the openings in the lamina fenestrata. A suggestion of a finer porosity is apparent in the original print in the thin tangential section of the lamina densa. In lower left corner may be seen the external surface of the lamina densa between the pedicels again showing a characteristic pattern which appears as minute depressions. Fixation as in Fig 5 X23 000

12 (Fig 12) and the mu marker, and in the strands of the lamina densa, itself. The diameters of these apparent holes vary from about 50Å to 200Å with an average diameter of 90-110Å. These diameters are difficult to measure with precision and the values can be given only as approximate ones. Although these diameters correspond to possible values for the pore diameters of the glomerular capillary walls suggested by Pappenheimer, et al (25) and others it cannot be stated that they represent openings of pre-existing filtration pores until it can be proved that they are not artifacts. To obtain this proof will be most difficult, if not impossible, with presently available technics

If some type of preformed porosity is present in living capillaries similar to that apparently visualized in the electron micrographs, then glomerular filtration involves three stages - Hall (26). First, direct access of the plasma, free of particulate bodies, to the lamina densa without passage through a cell membrane is assured by the relatively large openings in the lamina fenestrata. Second, the fine porosity of the continuous, thin, lamina densa serves as the ultrafilter permitting retention of the large non-filterable molecules (proteins) while allowing the fluid of the plasma with its electrolytes and the smaller filterable molecules to filter without differential permeability. Third, the pedicels, by remaining separate, maintain discrete, free areas of the external surface of the lamina densa which assure a maximal filtration rate with minimal filtration pressure and the shortest, least impeded pathway to capsular space for the glomerular filtrate. It may be suggested at this time, that any change in the area of the pedicels in contact with the lamina densa would increase or decrease the free surface area of the capillary wall and probably by this means influence filtration rates Such a process may take place with normal physiological or pathological processes There are, as you know, experiments such as those of Bayliss and Lundsgaard (27) which have been interpreted to support the concept that glomerular filtration rate does change independent of blood flow.

In support of the possibility that the apparent fine porosity may be related to a real porosity of the lamina densa the following may be said. A uniform fine porosity of such minute dimensions has been found only when the possibility exists that lamina densa (or lamina fenestrata) material has been sectioned Also, the apparent porosity is within the order of magnitude which fits generally held theories concerning pore size in ultrafilters with a permeability comparable to that of the glomerular capillaries. The apparent fine porosity may be a fixation artifact, or result from the removal of the plastic, or an artifact induced by some other process of the technic. Yet, Callan and Tomlin (28) obtained clear electron micrographs of uniform oval pores, about 300Å in diameter in frog oöcyte nuclear membranes without fixation, or embedding in plastic, or any other treatment other than washing and drying

(25) Pappenheimer, J R., Renkin, E.M , and Bonero, L.M 1951 "Filtration, Diffusion and Molecular Sieving through Peripheral Capillary Membranes: A Contribution to the Pore Theory of Capillary Permeability," Amer. J. Physiol., 167:13-46
(26) Hall, B V 1953. "Renal Filtration and Ultramicroscopic Structure of the Glomerulus," Federation Proceedings, 12:467-468.
(27) Bayliss L E , and Lundsgaard, E 1932 "The Action of Cyanide on the Isolated Mammalian Kidney," J. Physiol., 74:279-293
(28) Callan H G and Tomlin, S G 1950. "Experimental Studies on Amphibian Oocyte Nuclei I Investigation of the Structure of the Nuclear Membrane by Means of the Electron Microscope," Proc Roy Soc Lond. Ser. B. 137:367-379.

Our sections are regularly too thick to visualize the structure of the lamina densa itself in perpendicular section, but a few fortuitously thin sections give the suggestion that there may be a sponge-like structure in this membrane, with connecting irregular spaces. In fact, at times the larger net-like pattern of the endothelial cytoplasm appears to change into a progressively smaller and smaller pattern until it merges into the fine sponge-like pattern, sometimes apparent in the lamina densa. The intimate relationship between the lamina densa and the endothelial cytoplasm has been consistently and repeatedly observed.

Our observations on human material have not been extensive, yet they are sufficient to support the conclusion that the organization and fine structure of the human glomerular capillaries is essentially the same as those of the rat. Fig. 13 shows that the lamina fenestrata is present on the luminal surface of the lamina densa, and pedicels on the external surface In these sections the lamina densa appears thicker than it does in the rat but this may or may not be so in life, since the human kidney sections are difficult to interpret accurately. Their quality is inferior to the sections of the rat tissues, for as yet fixation of the human tissues has not been fully satisfactory.

Fig. 14 represents an electron micrograph of the lamina fenestrata, lamina densa, a few pedicels and perhaps a trabecula on the right. It demonstrates the basic similarity in the fine structure of the rat and human glomerular capillary basement membrane.

DR. FOX I wonder if Dr Hall could tell us how, if he made a section of a piece of cellophane in which certain things would go through, the pores would compare with the pores we saw in these sections?

DR HALL: We have not done this but there are two or three papers where something of this nature has been done. One dealt with the egg-shell membranes of the chicken, and the other dealt with collodion membranes fashioned under controlled conditions by Bugher(29). In general these pores were much larger than the 100Å openings which appear in some of our pictures of lamina densa and they were very irregular. The structure of the collodion membranes appeared in section as a sponge. It may be that there is a similar structure in the lamina densa with irregular connecting spaces. The only even, rounded openings that have come to our attention are these that we have looked at today, and those of Callan and Tomlin(28) which were around 300Å in diameter.

DR. FOX You say the cellophane pores are larger than these you have shown us?

DR. HALL· They were not cellophane but collodion membranes, but the pores were larger, much larger in most cases

DR. FOX Were they wet or dry membranes?

DR HALL: They had to be dry membranes because they couldn't be examined under the electron microscope otherwise

(29) Bugher, John C. 1953. "Characteristics of *Collodion Membranes for Ultrafiltration*," J. Gen. Physiol , 36:431-448.



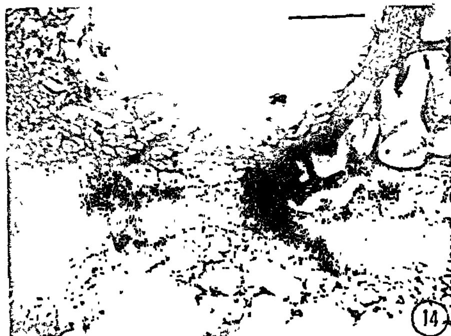


Fig 13 Electron micrograph of a human glomerular capillary showing lumen in center to top where there is part of an erythrocyte The luminal surface of the basement membrane shows the lamina fenestrata and the external surface shows pedicels Lamina fenestrata lamina densa and pedicels may be seen on far left, also Fixation alcohol-formalin-osmium X12 000

Fig 14 Electron micrograph of human glomerular capillary basement membrane sectioned tangentially and obliquely Lamina fenestrata lamina densa, pedicels and trabecula similar to that in the rat Fixation as in Fig 13 X20 500

MEMBER: There are different ways of preparing the membrane and of course the size varies greatly depending on how you dry it.

DR HALL: Yes, in these experiments the porosity was determined experimentally before they were examined.

MEMBER: The thing that I was wondering about is the question of artifacts. I know it is a very important process particularly where you do have these granular structures

DR HALL: That's right.

MEMBER: And I wondered whether or not the fact that you dry an open membrane and get pores in one instance, and in the second membrane do not find pores, is an indication that artifacts were not also present?

DR HALL: You are speaking of Callan and Tomlin's work now?

MEMBER: Yes, in drying the membrane you can dry a 14 film and get the same artifacts

DR HALL: But they found these pores in the nuclear membrane in the fixed, as well as the dried state.

MEMBER. But then do you not get artifacts because of fixation and precipitation just as you would with drying?

DR. HALL It is possible, and I would certainly be the first to admit that we cannot, and have not as yet, eliminated the possibility of artifacts in our own work The apparent fine porosity specifically may be an artifact induced by some process in the technic

DR HOLLIDAY Looking at the pedicels from above, would they appear like mountainous ridges, or finger-like processes?

DR HALL: Both In some regions they are free on the surface of the lamina densa and they would look like mountainous ridges viewed from an airplane, but in other views they could be obscured by the main central mass of the podocyte.

DR HOLLIDAY But one end of the ridge would ultimately be connecting with the podocyte, is that right?

DR HALL That is right The other end ending free, with a rounded, terminal swelling, (banjo-like), in a bay which is formed by a second order process (trabecula) which is parallel to the trabecula from which the pedicel arose, and the two pedicels, one on each side of the first pedicel, whose origin and terminus we are discussing, would complete the sides of the bay.

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DR. HOLLIDAY: Do you know anything about the connection between the pedicel and the lamina densa?

DR. HALL: In the better fixed specimens, there seems to be a very intimate connection down to 100Å units or less.

MEMBER: But at one point they appeared to be separated farther than that.

DR. HALL· In less well fixed preparations they seem to have pulled away from the lamina densa and also they tend to spread laterally and fuse so that the appearance of a second membrane lying on the external surface of the lamina densa is developed.

DR McCRORY: Is the lamina densa probably of epithelial origin?

DR. HALL: We can only give you our impression that it is not. Our opinion is based on evidence which was not stressed today. We have observed many times that endothelial nuclei and lamina densa material are intimately associated sometimes for nearly 36° around some nuclei

DR. McCRORY: This couldn't be like the nipping off of the eye on the potato -- that you have the nucleus pushed into the lamina densa

DR. HALL: Not always, certainly, for in other sections a rounded endothelial nucleus may project into the lumen on one side and on the other it can be seen to be within one-tenth of a mu of being in apparent contact with the lamina densa.

DR. GITLIN· Have you even been able to find the edges of these endothelial cells, where two endothelial cells would come together in the cytoplasm?

DR. HALL: Yes, but chiefly in the walls of the arteriole. There is one picture of an entering arteriole where it looks like the elastic lamina, or whatever it is that is comparable to the lamina densa there, actually terminates then continues. What seems to be undifferentiated endothelial cytoplasm goes through the small gap Nothing similar to this has been seen within Bowman's capsule

DR. EDER· What is the diameter of the pores in the lamina densa and how does it compare to the diameters of proteins?

DR HALL: In the best sections which probably have the least distortion, they average about 70 to 110Å units in diameter

DR. EDER: How does it compare with the dimenstions of other ultrafilters?

DR. HALL: It so happens that these resemble closely the dimensions which Elford (30) in England working with collodion membranes and ultrafiltrates estimated

(30) Elford, W J. 1937. "Principles Governing the Preparation of Membranes having Graded Porosities The Properties of 'Gradocol' Membranes as Ultrafilters," Faraday Soc Trans 33 (Part 2) 1094-1104.

they should be to filter in a fashion similar to the glomerular capillaries. To reply to your question specifically, the hemoglobin molecule, as I understand it, and somebody here can probably correct me if I am wrong, is cylindrical in shape and it may be 57Å in one direction and 34Å in the other direction. Now the apparent porosity is considerably larger than that, but Elford found that for proteins there is a factor which determines how much larger small diameter pores must be than the molecule which passes through them. This actor is sometimes as much as 2 or 3 depending upon the nature of the protein. Now it also happens that Pappenheimer (25) in his recent extended study of capillary function, I believe relatively independent of Elford's methods, and some of the other students of capillaries, also arrived at about 100Å as a possible figure for the effective pore size of glomerular capillaries We must keep in mind that effective pore size will probably be larger than mean measured pore size, as pointed out by Pappenheimer (31) If the pores are 100Å, as they appear to be, this would correlate closely with the theoretic and physiologic measurement.

DR KAPLAN Regarding the pore sizes in peripheral capillaries, Pappenheimer has said that uniform cylindrical pores of effective radius 30 to 45 A would account for the experimental observations on the passage of water and lipid-insoluble molecules at a population density of 1-2 x 10^9 per cm^2. (Physiol. Rev. 33·387, 1953.) The diffusion constant for mammalian glomerular capillaries is considerably greater than that for peripheral capillaries, about one-hundred-fold greater This does not mean that the pore size has to be so much greater in glomerular capillaries because the increased diffusion constant could be accounted for just as well by an increase in the population density Data on glomerular permeability, I think, would indicate that the sizes of glomerular capillary pores are not much larger than those in the peripheral capillaries and there is no inconsistency, then, between the observations of Dr Hall and those of the capillary and renal physiologists. The question of uniformity of pore size versus normal frequency distribution of pore size cannot be said to have been answered with any certainty Even in artificial membranes the decision appears to be a difficult one and there is a probability that there is considerable variation in pore size

The calculation of pore size from the diameters of molecules to which a membrane is permeable must take into account the fact that there is steric hindrance to the entry of molecules into the pore equal to the value $D' = 1 - \frac{a}{r}$ where a and r are the diameters of the molecule and pore respectively, In addition, there is a viscous drag opposing the rate of movement of a molecule through a cylindrical pore equal to the quantity $D = 1 + 2.4 \frac{a}{r}$ Then $\frac{D'}{D}$ represents the restriction to diffusion For example, when the radius of the pore is six times the radius of the diffusing molecule there may be as much as 50% restriction of diffusion If some of your pores are up to 100Å in diameter, Dr. Hall, this observation is still consistent with results of physiologic measurements.

DR HALL: To give the whole picture, as we must, so as to create no illusions, a number of our preparations show larger apparent pores, averaging maybe 50% larger diameters or more, though the measurements themselves are subject to considerable error. The technic is actually so difficult and uncertain, that it is unwise at this time

(31) Pappenheimer, John R. 1953 "Passage of Molecules through Capillary Walls," Physiological Reviews, 33 387-423

to accept the apparent pores as anything more than as suggestive of a possible real porosity existing prior to fixation and the other atrocities to which the membrane was subjected in obtaining its photograph. Of course, we know from several studies that have been made on rat proteinuria that large quantities of protein presumably filter through the rat glomerulus and the pores, if they are there, must be large enough to allow these proteins to pass through the lamina densa. It may be recalled also that changes in the level of testosterone or the level of thyroid activity or the level of protein in the diet have been shown to influence the degree of proteinuria of the rat. This raises the question whether these factors change the porosity of the lamina densa or is the change in proteinuria brought about by changes in other factors, such as the rate of protein resorption by the tubules.

DR. HEYMANN. How does the glomerular capillary differ in its properties or show up under the electron microscope when compared to other capillaries, say the capillaries of the mesentery?

DR. HALL. Our material includes some views of intertubular capillaries and they do not show a lamina fenestrata, nor do they have the pedicels on the surface.

DR. GITLIN: If you imagine these pores to be present in the cytoplasm of the endothelial cells, as the lamina fenestrata, it won't be necessary, as Dr. Pappenheimer has suggested, for protein to go through the interspaces, the spaces between the endothelial cells. In other words, the capillary wall let's say of the skin would be relatively homogeneous in effect to its filtration properties.

DR. HALL: That is correct. But, remember the holes which appear as prominent 1000Å openings making the endothelial cytoplasm appear as a network, have been seen only in the glomerular capillaries. The lamina fenestrata may very well be the structural feature which enables the glomerular capillaries to filter non-lipoid soluble substances and particularly the larger molecules at the very high filtration rate characteristic for them.

DR. GITLIN: Do you have any information as to the pore size in the skin capillaries with relation to pore size in the glomerular capillaries?

DR. HALL: Although our material may be interpreted as suggestive of a fine porosity for the glomerular capillaries, it has not shown a lamina fenestrata in the intertubular capillaries, as we have just mentioned. As far as seeing apparent fine pores is concerned, their demonstration depends largely upon getting optimum tangential sections. When starting with the glomerular study, it was reasoned that the glomerulus, with its small twisting folded capillaries, gave a maximum statistical opportunity to get thin tangential sections because there were so many twists and turns of the capillaries. That has proved to be true because we haven't had much luck so far in getting sections similar to them from intertubular capillaries.

DR. GITLIN. Do you think you would have a similar opportunity in the choriod plexus of the monkey? It would be very interesting to know. There must be porosity, of different sizes, but there must be porosity.

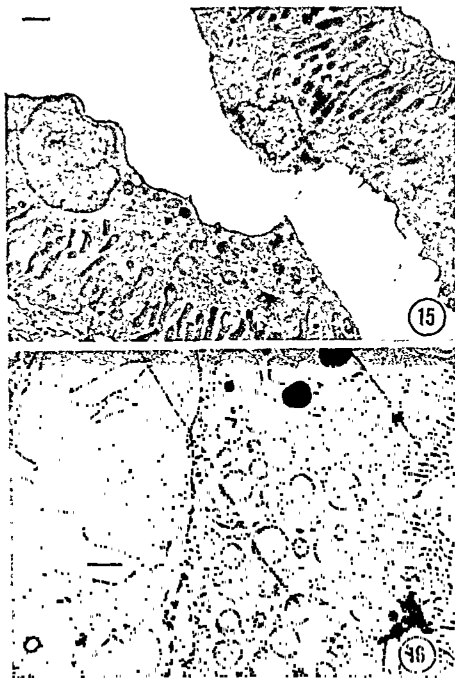


Fig 15 Electron micrograph of portion of distal convoluted tubule showing elongated mitochondria, intracellular membranes and a few microvilli projecting into the lumen Fixation 5% formalin buffered X8,000

[illegible]

basement membrane is usual and the fine cytoplasmic processes of the tubular cell at its base separated by communicating channels is commonly observed in these cells Buffered 5% formalin fixation X10 000

DR. HALL: Yes.

Dr. Metcoff has asked to see some illustrations of the tubules The few we have to show are largely of interesting sections that have been seen while searching for glomeruli. Our comments must be brief as we have not yet made a thorough study of even the limited tubular material we have.

Fig. 15 is a section probably through part of the distal convoluted tubule, showing elongated and rather irregular shaped mitochondria. In the absence of a brush border, one sees on the luminal surface a few small microvilli, somewhat like those pictured by Dalton(16) for the mouse. The nuclei project into the lumen covered by such a thin layer of cytoplasm that the photomicrographs of this cell give one the impression that poor technic has left naked nuclei projecting into the tubular lumen. The electron microscope shows what the light microcscope could not -- there is a thin, 0.1-0.2 u layer of cytoplasm covering these nuclei. The section is too thick and the fixation not optimal to show the internal structure of the mitochondria, but the presence of membranes lying on either side of the mitochondria is evident. As observed by Dalton, these membranes appear most numerous in the basal part of the cells. We agree with Sjostrand and Rhodin(32) in recognizing these as sectioned intracellular membranes, or specialized cytoplasmic sheaths in the sense of Pease and Baker.

Fig. 16 shows a capillary with erythrocytes lying in close contact with the basement membrane of a proximal tubule. Note the narrow waist of one erythrocyte and the close proximity of the capillary wall to the basement membrane of the tubule. We have been impressed by the absence of any considerable interstitial space in the kidney The luminal surface of the proximal tubule shows individual fibrils of the brush border The mitochondria in this preparation are rounded spheroids and give only a hint of the characteristic inner structure, or cristae, described by Palade(13). The cytoplasm of these cells is generally separated into smooth irregular strands by corresponding clear channels. These channels generally reach the basement membrane, as may be seen here, and in the next figure. They may also be traced less frequently to or near the brush border of proximal convoluted tubular cells, or the less highly specialized surface of distal convoluted tubular cells. In preparations like these, there appears to be more "interstitial" space on the tubular side of the basement membrane than on the capillary side. These spaces may be artifacts but they give the appearance of irregular, curving channels, which may represent a similar pattern existing in the living cell It is quite possible that there is a complex architecture in each tubular cell dividing it into similar and repeated functional units This complex convoluted tubular cell architecture is present similarly in both rat and human kidneys.

DR GITLIN May I ask a question at this point concerning the apparent structure in the red cells, that is, they also show that same granularity. Do you think that they are artifacts or actually exist?

DR. HALL This appearance has been observed a number of times in thin sections

(32) Sjöstrand, F S., and Rhodin, J. 1953 "The Ultrastructure of the Proximal Convoluted Tubules of the Mouse Kidney as Revealed by High Resolution Electron Microscopy" Exp. Cell Res., 4.426-456.

of the red cells. It is possible that some process in the technic removes a constituent of the red cells, normally present as a disperse system in these sac-like cells. The apparent porosity seen here is about 5 times as large in diameter as that seen in the lamina densa. In addition, these holes are quite irregularly shaped while the much smaller holes of the lamina densa appear as smooth ovals and circles.

Fig. 17 pictures parts of two kidney tubules, separated by a small portion of a capillary, the one on the right with brush border fibrils, the other with a few microvilli projecting in the luminal space. Note on the right the irregular mitochondria and prominent large clear channels which extend from the basement membrane to the brush border. On the left the mitochondria are more elongate and the channels are narrower and more intricate. Again it may be noticed that there is little or no interstitial space between the capillary wall and the basement membrane, but a very extensive system of channels ramifying through the cytoplasm of these cells, and perhaps between the cells. Membranes can be seen lining these channels and enclosing mitochondria, which show some structure, but not clearly. If the brush border and the mitochondria were not well preserved, one might regard these channels as pure artifacts, but since the channels appear regularly in well-fixed tissues, it is possible that they may be a part of the functional mechanism of the cells.

Fig. 18 is a view of a proximal convoluted tubule of another rat where open channels are not evident and the intracellular membranes are not prominent. However, the mitochondria are clearly represented as irregular, elongated bodies, showing transverse cristae. The brush border fibrils are not well preserved. The capillary wall in this instance may have been pulled away from the basement membrane, and what appears to be collagen fibers can be seen in the space between the capillary wall and the basement membrane.

Fig. 19 represents a typical view of a part of a proximal convoluted tubule of man, showing brush border, rather small mitochondria, and a prominent basement membrane. Some investigators have been unable to visualize the brush border in human kidneys, but our preparations show it clearly. Sjöstrand and Rhodin have restated Funke's (33) old suggestion, made for brush border of intestinal epithelium, by suggesting that the brush border of the kidney is formed of densely arranged cylindrical "ducts", closed at both ends, so that the cell surface facing the lumen is smooth and bordered by a single membrane. It is evident from this figure that the brush border appears here in the human kidney as a series of separate, rod-like fibrillae, as Pease and Baker, and Dalton, et al have described in the mouse. We have sections which give the same honeycomb-like appearance which Sjöstrand and Rhodin have shown but sections like the one represented by Fig. 20 lead us to support the opinion of Pease and Baker, Dalton and others that the brush border is composed of separate individual rods about 400Å wide and at least one mu long. Some sections give the impression the fibrils may be as long as two mu. The apparent tubular nature of the fibrils of this last section may be seen in both longitudinal and oblique or cross-sections of the individual fibrils. We are again hesitant to guess as to the exact nature of these structures in the living animal, for what we now look at is but a caricature of the living structure, exaggerated 38,000

(33) Funke, O. 1856. "Beiträge zur Physiologie der Verdanung. II. Durchgang des Fettes durch das Darmepithelial," Zeitschr. f. wiss. Zool., 7:315-327.



Fig 17 Electron micrograph showing part of a proximal convoluted tubule on right and part of a distal convoluted tubule on the left The intracellular channels are obvious and the interdigitation of the intricate cytoplasmic processes is somewhat similar to the complex of processes and spaces formed by the pedicels and trabeculae of the podocytes Buffered OsO_4 fixation X12 500

Fig 18 Electron micrograph of part of a proximal convoluted tubule showing elongated mitochondria with their internal cristae Intracellular membranes are barely visible Fixation as in Fig 17 X6 000





Fig 19 Electron micrograph of part of human proximal convoluted tubule showing well developed brush border small mitochondria and nucleus Connective tissue fibrils are intimately associated with the prominent basement membrane Alcohol-formalin-osmium fixation X6,500

Fig 20 Electron micrograph of brush border fibrils showing tubular appearance in both longisection and cross-section Buffered 5% formalin fixation X38 000

times. However, such pictures do suggest that the brush border is composed of individual fibrillae, which may be tubular, or whose central cytoplasmic core precipitates or is removed by the action of some process or processes in the preparation of it for viewing under the electron microscope.

This question of how nearly our observations represent reality, of course, is an old one not exclusively the bugbear of electron microscopy, but the difficulty of arriving at an exact answer to the question probably increases as some function of the greater magnification employed in electron microscopy.

II. Studies of serum and urine proteins

CHAIRMAN RAPOPORT: Dr. Kretchmer will now present material on protein and amino acid transport by the kidney.

Dr. KRETCHMER: I would like to discuss "The Tubular Handling of Proteins and Amino Acids."

Approximately 60 years ago Altman described various shaped granules which appeared in the cells of all organs and called them mitochondria. He noted that mitochondria took various stains. About 40 years ago Michaelis(1) discovered the Janus Green-B dye which was used as the specific mitochondrial stain.

In 1913 von Molendorff(2) described a situation existing in the kidney where after an injection of the dye, trypan blue droplets having the same blue color appeared in the cytoplasm of the proximal tubule cells. He called this phenomenon athrocytosis, and it is essentially of this phenomenon that I would like to speak today and to point out how "athrocytosis" is a misnomer in many ways.

In 1935 Gerard(3) studied the phenomenon of tubular absorption of colloidal dyes using the Necturus. This animal has a dual system of nephrons, one a closed nephron with a glomerulus, a proximal convoluted tubule, a thin portion, and a distal portion. The other nephron has a peritoneal duct, connecting to the proximal tubule What Gerard did was to inject trypan blue into the peritoneal cavity, which was taken up as droplets by the proximal convoluted tubule cells of both nephrons but with more droplets in the open nephron. If he injected trypan blue subcutaneously, then the trypan blue was taken up in equal amounts by both nephrons If he used a larger particle such as Prussian blue and injected it into the peritoneal cavity, then the droplets were found in the distal end of the proximal convoluted tubule of the open nephron, but not at all in the closed nephron Not only was there this distinction between the two dyes, one a smaller colloid, and one a larger colloid, but there was also the distinction of proximal tubule location of the formed droplets.

He went further in his studies and studied various sized substances and how they were taken up in the nephron as regards location in the proximal convoluted tubule.

Proteins such as ovalbumin, hemoglobin, and chlorophyll, were taken up in the middle third of the proximal convolution of the open and of the closed nephron. When higher molecular weight substances were used such as globulins, casein and large Prussian blue, the cytoplasmic droplets located only distally in the proximal convoluted

(1) Michaelis, L., "Die Vitale Färbung, eine Darstellungs methode der Zellgranula," Arch Mikr Anat 55, 558, 1900.
(2) Mollendorf, W.V., "Die Dispersitat der Farbstoffe, Ihre Beziehungen zu Ausscheidung und Speicherung in der Nier., Anat Heft ," 53, 81, 1915.
(3) Gerard, P , and Cordier, R., "Esquisse d'une Histophysiologie Comparée du Rein des Vertébrés." Biol. Rev., 9, 110, 1934.
(4) Bayliss, L.E Tookey-Kerridge, P. M , and Russell, D S , "The Excretion of Protein by the Mammalian Kidney." J Physiol 77, 386, 1933

tubule, and only in the open nephron circuit. This is analogous to the type of conclusion that Bayliss(4) arrived at in 1933. At that time he showed that substances with molecular weights about 70,000 could not normally pass through the glomerulus.

With this brief survey of the older literature we can better visualize what occurs in the rat, the animal utilized in this study. The histological portion of this presentation is taken in part from work done by Oliver and MacDowell and Lee (5,6,7). If the nephron of the rat is dissected from a 3/4 nephrectomized kidney there is noted a conglomeration of droplets in the middle third of the proximal convoluted tubule. These droplets are formed during the proteinuria resulting from the nephrectomy. If, instead of producing such a traumatic situation as a three-quarter nephrectomy, we produce an experimental proteinuria with an intraperitoneal injection of egg-white protein, we find large homogeneous droplets within the proximal convoluted tubule cells These droplets appear at the expense of the mitochondria or as the pathologist calls them, batonets, for as the droplets increase, the batonets decrease.

Thus, the animal is injected with egg-white; the egg-white absorbed by the proximal tubule cells, and the droplet configurations seen. These droplets sometimes appear to fill the entire cell and even obscure the nucleus. Dr. Oliver has given the name of "Mulberry cell" to this type of cell. The droplets are gram-positive whereas the mitochondria are gram-negative

There is a decrease in mitochondria and an appearance of large homogeneous droplets within the cytoplasm. Over a period of time they seem to replace the entire mitochondrial network The major point is that this process occurs in a normally functioning nephron, which is accomplishing the absorption of the protein from the luminal fluid

In order to indicate this more clearly, if approximately 25 mg of uranyl acetate is injected into a rat and three days later followed with an injection of egg-white, cellular degeneration and disruption of the tubule is seen but no droplets are present. Due to this we maintain that a normally functioning nephron, particularly the middle third of the proximal convoluted tubule, is needed for the absorption of the protein from the luminal fluid In acute renal failure where you see atypically regenerated cells, cells do not take up the protein Droplets result from the injection of egg-white and also these droplets appear after the intraperitoneal injection of other proteins, such as bovine albumin, rat serum albumin given to the rat over a period of time, and hemoglobin; in addition, the same type of droplets form after injection of amino acids.

In a sense, this complicates the picture and brings up the question of the simplified dynamics that athrocytosis implies. For here the same structural unit forms, both from injection of different proteins and also from amino acid injection. At the same

(5) Oliver, J , "New Directions in Renal Morphology, A Method Its Results and the Future, Harvey Lectures," Series XL, 1944-45

(6) Oliver, J "The Structure of the Metabolic Process in the Nephron.," J. Mt Sinai Hospital, 15, 175, 1948

(7) Lee, H , "Protein Metabolism in the Rat Nephron III Histochemical Demonstration of Amino Acids." J Exp Med., in press, 1954

time that droplet formation occurs, mitochondria decrease in amount as the droplets increase in number. It was the feeling of Dr. Oliver's group that the droplets were not *ordinarily flocculated protein within the cytoplasm*, or certainly not flocculated amino acid but rather that they were possibly a location of metabolic activity, and that the chemical constituents present in droplets might be derived from mitochondria. This conclusion was reached from the histological studies.

To quote Dr. Jean Oliver, "To summarize droplet formation, it is a feature of the metabolic process in the renal cell when the enzymatic mechanisms of disposal are at their maximum of development. It is quantative not qualitative, factors that account for the droplets. A very little egg-white or a large amount of serum proteins produce the same type of intracellular reaction. The intracellular phenomena of proteinuria, previously described under the separate categories of absorption, athrocytosis, hyaline droplet formation, and even as evidence of a general disturbance of the metaprotein metabolism becomes then an integrated and unified process"

The major questions which remain unanswered are: 1) what is the relation, if any, of droplets to mitochondria; and 2) can we agree, chemically, with the histologist that metabolism (either protein or amino acid), goes on within these droplets, in greater degree than in any other cell particulate.

The approach to the second part of the problem involves the cytochemical comparison of droplets to mitochondria The studies reported were carried out with the assistance of Drs. H. W. Dickerman, Francis Cherot, Helen Wells Rappaport, D. F. Klein, A A. Komninos. Attempts were made with two different techniques to isolate pure and unaltered droplets· 1) *cellulose column separation which was used by Dr Werner* Strauss; and 2) differential centrifugation.

With *the column separation* technique, a pure droplet can be isolated, but the fault is the continual pouring of the homogenate through the column, thus adsorbing certain compounds and washing out others The differential centrifugation technique, which we will go into in more detail in a moment, yields mixtures of droplets and mitochondria

In the technique of differential centrifugation, the kidney cortex is removed and homogenized with 0.88 M sucrose. In this *solvent the mitochondria maintain their rod* shape, whereas in isotonic saline they tend to swell. It is presumed that this hypertonic sucrose is probably isotonic to the mitochondria. The homogenate is spun at 600 times gravity *for 2 minutes* and two fractions are isolated. The "nuclei fraction" consists of a mixture of nuclei, unbroken cells, some red cells, and a few mitochondria. The "cytoplasmic fraction", also erroneously named, consists of the larger particles, microsomes and the final supernatant fluid, plus whatever soluble substances were extracted from the nuclei. The "cytoplasm" is spun at 20,000 times gravity for 30 minutes, from which is *derived the* larger particles and another supernatant fluid labeled Supernatant-2.

The larger particles from a control kidney, an animal which has not received an injection of egg-white, consist of fairly pure mitochondria, whereas from an animal which has *received an injection* of egg-white, the larger particle fraction consists of a

mixture of droplets and mitochondria. Various and sundry attempts to separate the droplets from the mitochondria met with failure except by using column separation, which is a much more tedious, and in many ways, a more erroneous technique. The larger particles derived from differential centrifugation were used with the knowledge that whatever values were obtained would be minimal values, at best. The Supernatant-2 is spun 20,000 times gravity for 90 minutes, or 50,000 times gravity for 60 minutes. A translucent amber colored pellet, termed microsomes, is obtained, and a final supernatant fluid This final supernatant fluid consists of material that was dissolved during fractionation, plus "clear cytoplasm".

Dr. Strauss(8) using column separation was able to isolate pure droplets and analyse them. He found that the nitrogen, phosphorus, phospholipid and nucleic acid content of the droplet was similar to that found in the mitochondria. He also showed that droplets isolated 14 hours after an intraperitoneal injection contained much less oxidative enzyme activity (estimated by the Thunberg method) than did mitochondria. Immunochemically, he showed that the droplets contained 6% of their dry weight as egg-white whereas the mitochondria contained merely 2% egg-white. Oliver has shown with histological and cytologic studies that the droplets vary as time progesses. In other words, after giving an intraperitoneal injection of egg-white or any other protein, including hemoglobin, one finds that three hours after the injection these droplets show heterogeneous staining properties This persists for about 12 to 16 hours after the injection At 18 hours, they become more homogeneous, and at approximately 40 hours they are distinctly homogeneously stained.

It was the feeling that this was an indication of two things: 1) of formed droplets, -- at least the homogeneity of the droplets was an indication of completely formed droplets, and 2) the non-homogeneous droplets, the heterogeneously stained droplets, were an indication of forming, young droplets. In the early hours after the egg-white injection we were dealing with an incomplete droplet. Later, after the egg-white injection, 18 hours and thereafter, we were dealing with a complete droplet.

In order to study this, cortex was ground and the various cell parts were isolated by differential centrifugation These parts were analyzed for succinoxidase and cytochrome oxidase activities and rated in terms of microliters per milligram nitrogen. The fractionation was continued over a period of 41 hours, and samples of the kidney were taken from animals which had not received the egg-white and from animals which were killed 3, 12, 18 and 41 hours after the intraperitoneal injection -- and the activity determined.

In the total homogenate, which is a reflection of all the particles within the cell, we find that at 12 hours post-injection there is approximately a 25% decrease in succinoxidase activity, but at 41 hours the control value is reached

In the "cytoplasm" fraction, which also contains droplets, there is again this decrease at 12 hours and the return to normal at 41 hours. The larger particle fraction was examined with the phase microscope throughout the period of the experi-

(8) Strauss, W, "Column Separation of Kidney Particulates," J Biol. Chem., in press, 1954

ments. It was estimated to have a droplet:mitochondrial ratio approximately 1:1 up to about 18 hours. At 41 hours there is a droplet fraction with less mitochondrial contamination.

The succinoxidase activity (Warburg) at three hours shows no decrease whereas at 12 hours there is approximately a 50% decrease in activity on either the wet weight or nitrogen basis. As the 41 hour period is approached, the succinoxidase activity returns to control values While this decrease is apparent, the microsomal and final supernatant fluid succinoxidase activities rise. The same type of study was accomplished for cytochrome oxidase, as a check on the succinoxidase to show that our entire system was in excess. We again showed this decrease in the droplet-mitochondrial fraction, and if not a rise, at least a constancy in the microsomal fraction and a possibility of a rise in the supernatant fluid.

This leads us to the conclusion that the droplet form is more dynamic than one would expect from a purely flocculatory phenomenon; that there is a type of metabolism that we are familiar with in mitochondria also occurring in the droplets. In addition, it was the feeling that in order for a mitochondrium to become a droplet, the mitochondria may have broken up and become microsomal in size during the formation of droplets. A similar conclusion was reached separately by Dr. Oliver during his examination of the histologic material. This is very difficult in many ways to coordinate with Palades' (9) electron microscopic examination of mitochondria wherein he shows a fairly rigid mitochondrial structure.

The droplet resulting from the amino acid is gram positive and Janus Green B positive, as are protein droplets, but does not appear as large or as abundant as the protein droplets In order to study the relationship of the droplet to the amino acid, l-lysine was injected intravenously and the lysine in the plasma and the amino-nitrogen in the cortex determined Initially, after the injection, there is seen a very high level of amino nitrogen in the plasma; this decreases within about 15 minutes to slightly above the control value and then there is a slow decline downward to the control value. In the cortex the results are fairly similar. As reported by Friedberg and Greenberg(10), the amino nitrogen content of the renal cortex on a wet weight basis increases about threefold after the injection of amino acid The amino nitrogen, per gram nitrogen, increases about twofold Both of these values follow the same pattern: a steady curve downward.

In an attempt to show that the lysine was concentrated in the droplet form, particles were isolated at 15 minutes post-injection It was found that all of the lysine appeared in the supernatant fluid. Thus, the lysine either was in the supernatant fluid and not in the droplet, or the lysine, which is a highly soluble amino acid, had been leached out of mitochondria or of the droplet wherever it had appeared. This decline in the amino-nitrogen curve was possibly an indication of incorporation of the lysine into a protein. If that were the case, particles should be isolated at 60 minutes after

(9) Palade, G., "The Fine Structure of Mitochondria, An Electron Microscope Study," J. Histochem and Cytochem., 1, 188, 1953.
(10) Friedberg, F., and Greenberg, D.M., "Partition of Intravenously Administered Amino Acids," J. Biol. Chem., 168, 411, 1947

intravenous injection of lysine. If the lysine were incorporated into the protein, possibly the protein would stay with the particle where it was formed.

The particles were isolated after 60 minutes and with trichloracetic acid the proteins were separated and removed from all other contaminants. The protein was hydrolyzed and the amount of lysine present measured by paper chromatography. The lysine content in the protein from the larger particles was approximately four times as much as in pure mitochondria. This was verified by utilizing a specific lysine decarboxylase (isolated from bacterium cadaveris) and measuring CO_2 evolved. *more lysine in the protein from the droplet-mitochondrial fraction than in the protein* from the pure mitochondrial fraction The amount of lysine, in a control animal's polyglot protein fraction, would be of the order of 2% to 4% of the wet weight. In the animal which had received lysine intravenously, the lysine content of the protein would be in the range of 8% to 12%. This would be a minimal value because of the mitochondrial contamination. By varying the dose of the intravenously administered lysine, and plotting content of amino nitrogen in cortex against dose, a curve is obtained with an increasing slope finally reaching a plateau. It is at this plateau that the first droplets are seen This was observed separately by three different observers and they all agreed that the droplets appear in large quantities when the maximal level of lysine or amino acid uptake by the kidney cortex has been either approached or reached

From the experiments it is concluded that tubular handling of protein is not an ordinary flocculation of proteins, but that both the protein and the amino acid absorbed are combined with certain chemical constituents of the cell. Presumably these chemical constituents are derived from the mitochondria, either directly or through an agency of a smaller particle, microsomal in size. There are problems which remain unanswered: 1) how a molecule as large as a protein gets across the tubule cell; 2) how does the protein return to the blood stream; in other words, how does it participate in the total economy of the organism. We have only preliminary experiments for this.

In summary, then, we feel that the term "athrocytosis" is an erroneous term; and that the droplet, which has been described for fifty years, is not merely a droplet of imbibed protein, but is a metabolic site within the cell, presumably for handling the absorbed protein or the amino acid.

DR METCOFF· Why do you think that these proteins, or amino acids, were meant to be absorbed from the lumen and not provided from the vascular side of the tubule?

DR KRETCHMER I think most of the historical data bears that out. Specifically, the work done by Bieter(11) around 1937, where he indicated that protein when exposed to an aglomerular tubule will not permeate the cells, but will only do so if a glomerulus is present

(11) Bieter, R N "Albuminuria in Glomerular and Aglomerular Fish," *J. Pharm*, 48, 407, 1931

DR. METCOFF: Do you have such data showing that they come first from the luminal side?

DR. KRETCHMER: No, we have no data, or that which we have is no good. There are the observations of Oliver where he says that early in the formation of droplets, the droplets are at the apical end of the tubule cell. Later on they are more diffused in the cytoplasm. But I don't think that we can use this as completive data.

MEMBER: Then do you have any observations on animals that have a severe spontaneous proteinuria of their own, not one due to injection of egg albumin, etc.?

DR. KRETCHMER: Yes. One sees these droplets in small amounts present in rats, before you do anything to them.

MEMBER: Well, are there larger amounts if you make the rat proteinuric?

DR. KRETCHMER: Yes, larger amounts if you make them heavily proteinuric.

It has been reported by pathologists that in human nephrosis you do not see what they call hyaline droplets. We would prefer not to call these hyaline droplets. Since we think they are more than hyaline, and more than flocculations. One sees droplets in both normal and abnormal tubules, and they've been called by all sorts of names.

MEMBER: Do you have any knowledge about the lipase activity diminishing, or increasing in the tubuli?

DR. KRETCHMER: No, we had plans to do lipase but never have gone into it.

DR. GITLIN: I think Dr. Kretchmer ought to be congratulated because these problems have been kicking around for an awfully long time. Dr. Mender tried to solve it with azoprotein and Dr. Herwitz tried to solve it with radio-activity; we have been trying to do it also, but by far less ingenious methods than have been described here. We have been using azoproteins and radio-active proteins in the liver, and not in the kidney, and exactly the same phenomenon takes place. The protein is picked up by another protein and is masked completely in the prosthetic group.

DR. KRETCHMER: Let me get that clear. You mean there were two reactive groups on your diagram?

DR. GITLIN: No, we use the protein itself. For the label we used the dye and the radio-activity of the iodinated protein We tried to pick these proteins up immuno-chemically, as well as histologically, since histologically they don't seem to react as the mitochondria do. Immuno-chemically the proteins are not recognizable as the original proteins that were injected. They are coupled in such insoluble form that you can pick up the label in the large particle fraction and can actually pick out the individual particles. These particles do not react as mitochondria and do not react immuno-chemically.

MEMBER: This albumin is a peculiar thing; when you give it to mice, you get non-protein isotope in the urine, in large quantities.

DR. GITLIN: You're right, that just simply indicates again that you have got a metabolic process going on, the protein being coupled, converted, and the radio-active iodine being excreted.

DR. KRETCHMER: We've attempted to show an arterial venous difference between the renal veins and the aorta, after hemoglobin injections, and all our attempts to far have met with failure. We have shown an arterial-venous difference about 54 hours after the hemoglobin injection, with the arterial side higher than the venous side. We have not shown the venous side higher than the arterial side. I think it needs finer methods than chromotograph which we are using. Another interesting thing which make the tubule cells unique, Rathner reported that after hemoglobin injections, the hemoglobin is taken up by the tubules, and there has been tremendous concentration in the plasma but it has not been taken up by the cup cells, or actually any of the periphery endothelial cells.

MEMBER: I wonder if you would state how you determined the albumin on the droplets immuno-chemically. Dr. Gitlin says that in the case of liver there was a complete masking of the specificity of the antigen that he studied, and in the tubules you found that not to be the case.

DR. KRETCHMER. Well, this was work done by Dr. Werner Strauss, and as I know it, just cursorily, he developed a complement precipitating test for the egg-white proteins and used that against his purified droplets

DR. GITLIN Well, how did he get his droplets into solution to get a precipitating test? We did it by an absorption means, make the anti-serum in the droplet, and if you get absorption of the protein onto your droplet, it takes out a certain amount of antibody from the antiserum We have tried to throw these droplets into solution but it is impossible to throw them into solution by any of the physical methods we have used.

DR KRETCHMER. He isolated his droplets on a column, and then dried and ground them Presumably he got a fairly good solution or an extract of his droplets.

MEMBER They agglutinate badly when you put them in serum.

MEMBER Do you remember which amino-acids behaved this way and which did not, in your experience, or in Oliver's experience?

DR KRETCHMER Well, in our experiences we agreed in several instances. We differed with regard for tryptophane, for example, which produced good droplets but turned over to kind of an amino uretic acid, very rapidly. The best droplets are formed by the basic amino acids, arginine, lysine, and histamine. The aromatic amino acids, tryptophane, glutamic acid, and cysteine produced very fine droplets. Oliver did histochemistry on the droplets produced by the various amino acids. He used histochemical methods, staining for end-amino groups, amino groups and sulfhydryl groups Dr Barnett and I saw the pictures, for example, with cysteine which showed

beautiful histochemical intensities within the droplets. All the amino acids produce drops. It's a semi-quantitative difference.

DR. METCOFF: Would it be possible to relate these observations to the use of protein hydrolysates intravenously?

DR. KRETCHMER: Henry, would you care to answer?

DR. BARNETT: The implications of this question are interesting but I know of no data which would help answer it. If one translates these data to the terms of kidney physiology, one could say that with increasing doses of lysine, the amino acid nitrogen content of the cell reaches a maximum which looks like a T_m. Presumably infusing amino acids at a rate which exceeded this T_m would lead to excretion.

DR RAPOPORT: Wouldn't it be much easier to correlate this with known clearances for individual amino acids?

DR. KRETCHMER Yes. We have some observations in the dog. I don't think we have any in the rat. For example, I think it was Pitts who showed that if he gave arginine, lysine was not absorbed. The spot in the chromatogram above the lysine spot in the injected animal is arginine. We have no explanation for this. The exploratory work that Handler at Duke has done shows that with infusions of amino acids, many amino acids are excreted other than those infused

MEMBER: *Didn't Dr Oliver also show that when he gave hemoglobin he could* block the absorption of bovine serum albumin from the tubule?

DR. KRETCHMER: Yes. That was the work of Lippman, Ureen and Oliver(12). *Oliver prepared the histological material and Lippman worked out the clearances. They* gave bovine albumin which formed bovine albumin droplets; they then gave hemoglobin *and formed hemoglobin droplets. If they gave hemoglobin to the* control animal alone, they found that the hemoglobin in the droplet form in the non-bovine albumin animal *was much greater than in the* bovine albumin-prepared animal.

In addition, instead of the curves running exactly parallel in the control animal which had received hemoglobin as against the injected animal which had received albumin plus hemoglobin, they ran so that they intercepted down at the X-axis, indicating, at least to Lippman, that there was a decrease in the absorption and an increase in the glomerular permeability to the hemoglobin.

MEMBER· Did you ever try any other substances, such as Dextran?

DR. KRETCHMER: Oliver has tried dyes, trypan-blue, and things like that. But I don't have any knowledge of polysaccharides.

(12) Lippman, R W , Ureen, H.J , and Oliver, J., "*Mechanism of Proteinuria* III, A Comparison of the Functional and Structural Aspects of the Effect of Certain Intraperitoneally Administered *Proteins on Hemoglobin* Excretion of the Rat." J. Exp. Med., 93, 325, 1951.

DR LANGE· This may be only of academic importance, but the Swedish authors have shown that when you have these droplet formations, lipase activity of the tubules decreases very markedly. This lipase activity may be, in turn, connected with the elevated cholesterol that we have in nephrosis, so therefore the poisoning or damage of the tubules by the albumin that leaks through may be the cause for the elevated cholesterol in the blood, via a decreased lipase activity in the tubule cell. We know that there is an almost straight-line relationship between the albuminuria and cholesterol, and that there is a slight lag of the one behind the other.

DR KRETCHMER Well, it is our feeling that this droplet formation is not an abnormal phenomenon but rather a markedly normal phenomenon. The question of cholesterol in the tubule versus protein in regard to droplets, it has been shown by earlier workers who studied lipid droplets within the tubules as against dye droplets, or protein droplets, that the lipid droplets are more distal in the tubules than are the proteinacious droplets. And this is supposedly when the pathologist looks at the slide and sees the double refractile body and says that this is cholesterol. To my knowledge, and I haven't gone through the literature, but there has been no intracellular analysis of the lipids during, say, nephrosis.

MEMBER· In support of the point you make that this is probably a normal process, is the fact that if very large amounts of hemoglobin are given intravenously over 90% of the hemoglobin given did not appear in the urine despite massive hemoglobinuria. In experimental hemoglobinuria, as much as several hundred grams have been known to be released at one time and no damage is seen to occur and only a few grams of this, one or two per cent, appeared in the urine.

DR KRETCHMER There is a little paradox in hemoglobinuria, however.

At Rochester they have shown that if you give monkeys a large dose of hemoglobin, you get quite a bit of it excreted in the urine, and you can figure that about 20% was reabsorbed by fairly rough ion analysis. If you give small doses of hemoglobin over a period of three days, a large amount is excreted in the urine, and it figures out that only about 10% of the dose is reabsorbed, so that it seems sort of paradoxical that in the second case, you have more of a chance to reabsorb and form the droplet, if you will, or break down the droplet and return whatever part of the globin molecule was returned to the blood stream and handled in that manner. They offered no explanation, and we have none, either

DR. METCOFF When one finds proteins in the urine having a molecular weight larger than, say, 150,000, how do they get there?

DR KRETCHMER· I don't know. Rigas and Heller (13) took 72 hour urines and measured the albumin, globulin, and total protein. With reversal of the $\frac{A}{G}$ ratio, they got about twice as much globulin as albumin. The electrophoretic patterns as reported by them in normal urine shows also very little albumin. The salted-out globulin appeared to coincide with the figures on an electrophoretic basis

(13) Rigas, D , and Heller, J , "The Amount and Nature of Urinary Proteins in Normal Human Subjects " J Clin Invest , 30, 853, 1953

MEMBER: Have you found that the proteinuria of exercise results in more globulin than albumin in the urine? The normal plasma pattern is not reproducible here.

DR. KRETCHMER: Well, if you consider, then, this curve or that one, or what Dr. Lawson has mentioned, the only real way you can explain this is an absorption of the albumin, because the other way you would be hard put to explain the globulin getting through, and not albumin, yet you assume, then, that it passed through as a plasma pattern in a ratio of approximately 2:1. Then the albumin should be twice as much as the globulin In this case, it is half as much. Therefore, I still think the only way you can explain it is by preferential tubular absorption of the albumin, as against the globulin.

MEMBER· You get exactly the same result in the casual urine. It doesn't have to be after exercise?

DR. KRETCHMER Yes --

DR BARNETT· Assuming that proteins pass through the glomerulus roughly in relation to their molecular weights, Bull(14) has calculated that in order to account for the quantity of globulin in the urine, something like 15 grams of albumin would have had to be filtered and reabsorbed

MEMBER Isn't it possible that there is less globulin, proportionately, being filtered?

DR. KRETCHMER: As a matter of fact, I think he has made this calculation on the basis that if they came through in proportion to the molecular size, then one would expect a certain ratio in the urine which was different enough from this; that it could be accounted for by reabsorption of 15 grams per day of albumin

MEMBER: Those are minimum figures?

DR. KRETCHMER Yes.

MEMBER: They are certainly conservative amounts as compared to the figures we have now where our filtrate is too low, and it is entirely possible that all the plasma proteins are being absorbed, so the figures you get are an absolute minimum.

DR. KRETCHMER: Yes

MEMBER: How big is a Bence-Jones protein?

DR. KRETCHMER· About 30-odd thousand.

DR. BARNETT· Has the urinary protein that occurs physiologically in the rat been analyzed in terms of the relation to the concentration of the serum proteins?

(14) Bull, G.M., Special Lecture Cornell U. Med. Coll., 1953.

DR. KRETCHMER: Yes. It's been analyzed by the group in California, by Sellers(15), and they have found more globulin also, more globulin than albumin.

DR. LAUSON. It is possible that normal proteinuria might be due to momentary breaks in the glomerular wall through which tiny spurts of whole plasma escape.

DR KRETCHMER: Yes, we can't just say some absorption; there's a whole lot of it, if it were pure plasma protein coming through.

DR METCOFF: I wonder whether a protein has ever been found which is too large to be consistent with any conceivable size of pore in the membrane For example, the polysaccharide chemists, I am told, have found polysaccharides in the urine of molecular size which are much too great to be present by any process of filtration that can be conceived of They presume, therefore, that these particles must penetrate through, or between, cells, perhaps being split off of tubular cells. Moreover, the question arises, how do such large particles as bacteria get into the urine? Do they penetrate through pores? Do they rupture through cells, etc ? Is it necessary to assume that filtration of very large molecular weight proteins has occurred, or have they perhaps been secreted by the tubule?

DR KRETCHMER: I feel this, that all the evidence that has gone before shows, if we can accept it, that the proteins are not secreted through the tubules, that the glomerulus is necessary for the droplets to form, which are evidence of protein absorption.

DR EDER. Further evidence against tubular secretion of protein is that the protein content of renal interstitial fluid is low

DR LAUSON There is another argument advanced by Chinard whether there is bulk transfer of filtrate, or whether a diffusion process is going on. I don't think we have to be stymied by a concept of a pore which doesn't fit the facts. I think it's about time we re-evaluated the situation to consider whether there is a filtration process, or whether there are not diffusion factors operating which would let us out of this impasse

DR PRESSMAN. I don't think the situation demands pores in any sense. The old sieve theory of membranes is really a 19th century theory. We know that substances get across membranes where we can't see the pores. In addition we know that the cell membrane is not a sausage packing It is not adynamic, but rather is very dynamic It contains ATP, and other energetic components used for the transfer process. For example, we know that in terms of pores, we have got to explain how glucose cannot get across a cell membrane as such It can only get across as a glucose phosphate, phosphorylated at the cell membrane. These phenomena occur without invoking something, in a sense, so ancient as pores

(15) Sellers, A , Roberts, S , Rask, I , Smith, S , Marmorsten, J , Goodman, H., "An Electrophoretic Study of the Urinary Protein in the Rat," J. Exp. Med , 95, 465, 1952

But it is very important to remember that these considerations of pore size are usually derived by the migration of molecules of known size through them. For instance, treating the molecule as an uncharged block, you can calculate, statistically, the chance of a molecule of a certain shape, going through a hole of a certain size. But the charge, both of the protein making the pore, and the protein of the molecules streaking through, are extremely important. For instance, membranes can be made which will permit simple anions to pass through, but not the cations. And these can be made with a great deal of selectivity, and they may well be with specificity, as to which charges are the protein or actually the type of proteins that a pore is going to permit to pass through. A pore will only accommodate a molecule of an appreciably smaller size than the pore, and there may be a good deal of differentiation and selectivity as to which molecules are permitted. I can well picture a pore permitting a globulin to go through, and not an albumin to go through, which would give the very picture you have here.

MEMBER: From the diffusion experiments that have been done with capillaries, and with the small bore tube, there haven't been enough discrepancies between the equations and the observed results to invoke electrically known forces.

MEMBER. How are the sizes of the pores measured in the membrane?

MEMBER: They are based on small capillary equations.

DR. PRESSMAN And these equations break down. That is just the reason why you are interested in the molecular size when you are having molecular dimensions of 50, 100A, etc. I believe that Stoner has prepared charged membranes which permit an anion to pass through, and not a cation The charges are extremely important.

DR KAPLAN: It should be pointed out that in artificial membranes pore size has been calculated from four different and independent sets of measurements. Regardless of the method of calculation and the observations on which the calculations were based, the answers are all in remarkably close agreement. The methods depended on
1) The calculation of pore radius (r) from Poiseuille's law, the D'Arcy diffusion coefficient and the dry-wet weight differences of the membranes.
2) The determination of r from the equation $P = 2\gamma/r$ where P is the critical pressure required to cause a barely perceptible flow of fluid through a capillary tube filled with a second fluid (immiscible with the first) and where γ is the surface tension at the interface.
3) The restriction to diffusion and filtration of molecules of graded sizes through isoporous membranes taking into account steric hindrance and viscous drag.
4) Electron micrographs of microscopic sections of calibrated collodion membranes made by evaporating silicon monoscide normal to the specimen surface.

It is very important to bear in mind that these measurments are made using uncharged particles and that remarks about reactions between charged membranes and charged particles do not apply to the measurements of pore size as elaborated by Pappenheimer

It must be remembered, too, that the pores under discussion are probably intercellular. Lipid-soluble molecules can diffuse through capillaries at rates which indicate

that they are probably diffusing through the cell walls themselves. Taking all these considerations together, the pore theory is still remarkably attractive and I personally think it would be very unwise at this meeting to throw it out summarily. (Laughter)

DR KRETCHMER: I agree.

CHAIRMAN RAPOPORT: I think we have about reached the time that we figured this discussion would take. We will start this next session with a paper dealing with some clinical chemical applications of electromigration in paper, by Miss C.A.J. von Frijtag Drabbe.

MISS von FRIJTAG DRABBE Two kinds of information are required for evaluation of changes in plasma proteins in disease; first of all, a knowledge of the qualitative changes, such as the appearance of abnormal proteins -- for example, the type associated with multiple myeloma, and second, an accurate measurement of the amount of proteins present in the several major fractions. The advent of paper electrophoresis offers new possibilities for obtaining such information

The equipment used for separation of proteins on paper has differed widely in design, and already a variety of techniques have been described. Among the simplest is that of Grassman, Hannig and Knedel.(1) Their special contributions in the field have been the introduction of a dye, amido Black 10-B, and a photoelectric densitometer for direct scanning of the paper strips.

[illegible] ig and Knedel (Fig 21) is composed of a chamber of [illegible] the central part of the chamber. Platinum electro [illegible] ompartments at the side and enclosed in a baffle system The power supply yields a direct current which can be regulated between 80 and 110 volts We found it most advantageous to work at 100 volts The power supply has connections for three chambers.

The buffer compartments are filled with buffer to exactly the same level after the bridge is inserted Barbital buffer of pH 8.6, and an ionic strength of 0.1 is used. Paper strips of 30 cm. by 4 cm are stretched over the bridge after they have been soaked in the buffer and blotted. One strip is applied to each side of the bridge.

The bridge is inserted in the apparatus, and the apparatus is connected to the current supply At present, we leave the current connected for about five hours before we apply the serum. About 8 ul of serum are applied on a transverse line onto the paper strips The proteins are allowed to migrate at 100 volts, for 16 to 18 hours, at a temperature of 10° C The strips are subsequently stained in amido-black 10-B. The several fractions of protein appear as blue zones in the paper. The different proteins have an approximately equal affinity for amido black which as you may know, is not the case with the other dyes like bromphenol blue when these are eluted

After the strips have been stained they are clarified in a mixture of alpha bromonaphthalene and paraffin oil which makes the paper translucent. The paper strips so

(1) Grassman, W , Hannig, K., Knedel, M , Deutsche Med. Wchnschr. 11, 333, 1951

clarified are sandwiched between two very thin glass plates, inserted in an aluminum frame, and passed through the densitometer, by means of a pinion and detent at one millimeter intervals over a light slit which has a width of one millimeter. The color density of the different protein fractions is measured by means of a selenium cell and a milliammeter. The milliammeter readings multiplied by 100 are plotted on millimeter paper on the ordinate against the distance in millimeters on the abscissa.

Fig 22 is a schematic diagram of the same apparatus that you just saw. The chamber is closed with a glass lid so that an atmosphere saturated with water vapor is maintained. This space is shallow and, since the apparatus is inserted in the refrigerator, permits a low temperature, of about 10°, to be maintained.

With an apparatus made in this country,* the voltage range that can be obtained is greater than that of Grassman and Hannig. At 300 volts runs may be completed in 5 to 6 hours, if an ionic strength of 0.05 is used, working with the same buffer of sodium barbital, sodium acetate and hydrochloric acid of pH 8 6.

Fig. 23 shows a typical normal serum pattern This pattern closely resembles that obtained from the Tiselius moving boundary method. Gaussian curves are completed to the base line. The base line represents the background color of the paper after the staining. I didn't tell you enough about that, I think. To stain the pattern an amido black 10-B saturated solution in methyl alcohol, with 10% acetic acid is used. After staining for 15 minutes the paper strip is destained to get the excess dye out of the paper by passing the strip through subsequent vessels of methyl alcohol with 10% acetic acid. The protein holds the stain, as you can well see here, and the paper gives it off. The paper never loses the blue color completely, but fades to a very pale blue. The densitometer is set to zero with the background color of the paper, and that will be represented by the base line

From the completed Gaussian curves, the surface area of each component can be determined quite simply by means of a planimeter.

Now you will see several pathological patterns. A patient with a perinephric abscess had a high gamma peak. Now if you keep in mind what this gamma peak looks like -- it is rather broad -- you can easily see the difference from this pattern, which is from a patient with multiple myeloma.

The myeloma pattern is characterized by a very high, very narrow gamma globulin peak

One also can demonstrate with paper electrophoresis the absence of certain fractions. Fig 24 represents the serum of a child with agammaglobulinemia. The child has no gamma globulin that we can demonstrate or that we can make visible on paper. As you can see we have here albumin, Alpha$_1$, Alpha$_2$, and Beta globulin. The gamma globulin is missing.

*Biophysics Instruments, Inc. Available through Arthur H. Thomas Co., Phila., Pa.

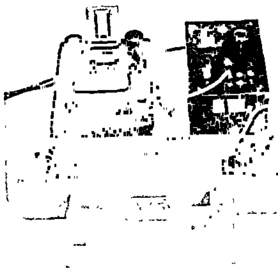


Fig 21 Paper electrophoresis apparatus of Grassman Hannig & Knedel

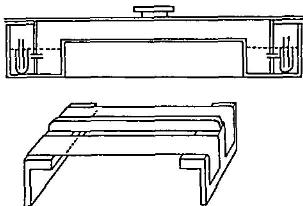
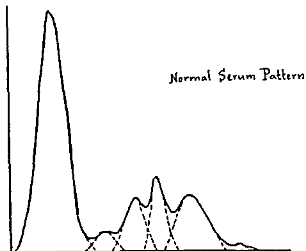
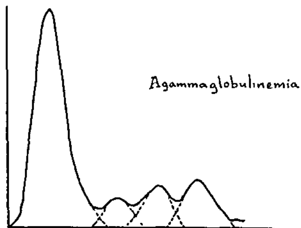


Fig 22· Schematic diagram of apparatus shown in Fig 21





Figs 23 & 24· Typical resolved paper electrophoresis patterns

It is also possible to demonstrate multiple components in each fraction. Here, one can see distinctly two components in the Beta fraction. Generally speaking, asymmetry in the Gaussian curves indicates the presence of more than one component in a protein fraction

In this pattern of terminal renal disease, multiplicity is even more obvious. It shows a very low albumin. Alpha-2 is high and the Beta globulin component has several sub-fractions Now these are simply sketched in, so that we know we have multiple components There may have been more, we don't know. When we measure the surface areas of such patterns, we simply measure the total Beta fraction, and we specify that there is more than one component, but we do not measure them separately because it would be impossible to do it reliably.

We were interested in seeing what the precision for these methods was and for that we determined the standard deviation of the differences between duplicates of a large number of patterns The results of these calculations show that the precision is slightly superior to that of the moving boundary method, and definitely superior to the salting-out procedure

We wanted to see how small we could make the samples and still get a reproducible result, while getting a pattern comparable to that which we obtained with the larger amount we usually work with This is a sample, with normal protein, from a healthy individual A pattern was made with 8 microliters of serum. Then we repeated this with 5 microliters, and 3 microliters, and then with 1 microliter. When we used 1 microliter, the albumin peak could be measured easily, but the other fractions were hazy and couldn't be determined. The comparison is quite good for the 3, 5 and 8 microliter samples

Analyses of known mixtures of albumin (Red Cross) and purified gamma globulin (Squibb) were performed. Comparison of the albumin found with that present in the mixture shows close agreement It is noteworthy that an excellent recovery of a small amount of albumin was effected in the presence of a high concentration of gamma globulin. The recovery of gamma globulin was satisfactory when amounts comparable to those ordinarily encountered in serum were tested. However, when a small amount of gamma globulin was combined with a relatively large amount of albumin, losses in recovery occurred

Recoveries of gamma globulin and of albumin added to the serum of a patient suffering from tuberculosis seem to indicate that interaction occurred in this serum, causing the recovery figures to be somewhat low.

A different result was obtained when the serum from the child with agammaglobulinemia was mixed with additional gamma globulin (Table 1).

Perhaps it is significant that the recovered gamma globulin exceeded the added gamma globulin by an increment that was the same regardless of the amount added. We have found that as little as 10 micrograms of protein can be detected by the amido-black staining technique Thus it is not an undetected moiety in this serum that is responsible unless it existed in combination with other proteins and was released only

when sufficient additional gamma globulin was added.

Table 1

Serum		Gamma Globulin	
Total Protein	Gamma globulin	Added	Recovered
530 ug.	nil	9.4 ug.	nil.
470 ug.	nil.	19 ug.	64 ug.
480 ug.	nil.	56 ug	103 ug.
660 ug.	nil	101 ug.	144 ug.

We then went on to compare the Tiselius moving boundary method with paper electrophoresis. This has been done before by quite a number of authors and with different success, depending upon what dye they used, what instrument, and upon the method There are many ways for making paper electrophoresis patterns, and different dyes for staining, and different ways of evaluation: by direct densitometry or by elution These variables make a big difference in evaluation of comparative studies. For instance: the accepted view is that bromphenol blue comines with less gamma globulin than does albumin. That may not be entirely so. It may be partly an artifact due to faulty elution.

We took serums on which Tiselius patterns had been made, and used them for running paper patterns Now as you all know, the Tiselius may show Beta anomaly, and also may measure lipid and carbohydrate in the Alpha globulin fraction. Therefore one would expect to find some difference in the paper values, since we don't have these sources of error in paper electrophoresis. We found that if we corrected the Tiselius pattern in a ver arbitrary way for the Beta anomaly, and compared them with 21 normal sera run on paper, we found good agreement, except for gamma globulin. We consistently found higher gamma globulin. Other authors have found the same.

We noted that albumin is partly absorbed by the filter paper strip, giving what we call a trailing effect, by taking purified albumin, in different concentrations, and making runs using different application lines.

We found that in serum patterns the same thing happens, although to what degree, we don't exactly know This we try to minimize by selecting our application line close to the center of the paper Naturally, since the gamma globulin peak is wider than that of any other component, the effect of trailing on the gamma globulin would be greater.

To obtain standard values we have studied a number of healthy males and females and determined the values for the different fractions. Though we still do have a higher value for gamma globulin, than that found with the Tiselius, comparisons are always made with the normal values worked out for this instrument. It is of no clinical importance.

I think that about covers it. If you have any questions you would like to ask, I would be very glad to answer them.

MEMBER: I am wondering about the accuracy of the densitometer readings, for instance, if you put a progressively increasing amount of proteins on a given piece of paper, stain it, and put it in the densitometer, do you get a strictly linear relationship between the densitometer readings and the quantity of protein placed in one area.

MISS von FRIJTAG DRABBE: Right But only in case there is no migration do you find a linear relationship

MEMBER: Even if the spot is quite dense?

MISS von FRIJTAG DRABBE. Yes, but with the following reservations. If your densitometer has a scale division running from zero on one side, to 200, if it is a logarithmic scale, readings higher than 100 are not reliable, so there are limitations. If you don't make a run, but simply apply serum and stain it, it can be shown that the relationship is linear, but it is no longer so, if you make a run. We have tried that by taking different amounts of protein and allowing them to migrate. The total surface area, though roughly proportional to the total amount of protein present, no longer shows a linear relationship

DR GITLIN: I'd like to point out one thing, though, as to the measurements here, and that is about agammaglobulinemia. I think the absence of gamma globulin on an electrophoretic pattern whether it is a moving boundary or paper strip is not necessarily indicative of agammaglobulinemia.

MISS von FRIJTAG DRABBE I agree with you because as you can see on the pattern where we had applied one microliter of serum, we knew very well that the other fractions were still present, but the instrument and the paper didn't indicate it any more So this child may have had some gamma globulin. Furthermore, he is being treated with gammaglobulin and we sometimes see a very vague zone of gamma globulin, but it is never what you would theoretically expect. So there is a certain bottom limit to the capacity of the instrument to indicate protein The child gets very little gamma globulin He gets 10 ml. of regular gamma globulin solution intramuscularly at a time Now, that diluted in the total plasma volume could hardly be enough to give you very much change in your pattern Still, we do sometimes find some.

DR GITLIN It should give you about 1-2% gamma globulin But you added 9 micrograms to 500 That is more than 1 1/2% of gamma globulin In the normal patient that would be 100 mg per cent If it is not aggamaglobulinemia you still won't detect the gamma globulin

MISS von FRIJTAG DRABBE: Yes, that's right. Well, that was a peculiar thing anyway, because when we added more, we didn't recover any. There may be some interaction between albumin and gamma globulin, which causes that.

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DR GITLIN Well, Armstrong showed that there was interaction between all the proteins. So that the Tiselius and paper electrophoresis are crude instruments, at best.

MISS von FRIJTAG DRABBE: That is very true, they are both crude instruments. They are helpful, certainly, and they are of value definitely, but we have also to consider that there are limitations to them.

DR. GITLIN: The absence of a gamma peak means that the gamma globulin is below the sensitivity of the method. The only time we like to make a diagnosis of "agammaglobulinemia" in a patient is when it runs below 25 mg. per cent.

MEMBER: Well, is it a fairly effective screening measure, or not?

DR. GITLIN: No. It is a good screening measure if you've got it set up, but there are other better screening methods that are applicable. You can do it clinically, I mean, but I don't think we ought to go into a discussion of agammaglobulinemia here.

MEMBER: Do you ever find such a pattern in an individual who has no gamma globulin?

DR. GITLIN: Yes, that is exactly what we were referring to, particularly in the age group between four and twelve weeks of age.

DR. SLATER: I think the work that has been presented this morning is very worthwhile. There are certain limitations that have been pointed out, and I think it is well that they have been emphasized. May I ask how much influence you think trailing caused by protein adsorption to the paper plays in the interpretation of your patterns? Certain studies have been done to decrease the adsorption phenomenon. Our experience has been that in separations such as the ones illustrated by Dr. von Frijtag Drabbe a constant adsorption of albumin occurs. If one develops a paper pattern in which only a small amount of albumin is present, as with a patient suffering from nephrosis, a much higher percentage of the total albumin is adsorbed than occurs when higher concentrations of albumin are present, as in a normal serum. This is one limitation which must be considered in interpretation of data.

The comparison between paper and free electrophoresis data of normal serum appears very satisfactory. However, in analyses of sera in disease states I think that errors can creep into the interpretation of quantitative date which would not be apparent at first hand. I wonder if that would be your impression also?

MISS von FRIJTAG DRABBE: Yes, but we have tried to eliminate that as much as we could, as I told you, by establishing our own normal values -- which give us already a lower albumin - at least slightly lower than the Tiselius would. It is not much, because we have been basing our calculations on equimolar up-take of dye, which may actually not be equimolar. However, using the instrument strictly for clinical work, if we get, say, a typical nephrosis pattern, or a typical myeloma pattern, that is, for the moment, all we can and all we want to do.

For two years we have been following the child with the agammaglobulinemia who has a history of constant and repeated infections until he was treated with gamma globulin. It is remarkable how little change there is in the different fractions, if at

different time intervals electrophoretic patterns are made of the serum on the same patient. As far as consistency goes, the tool seems to be quite reliable, but I do admit that it has some shortcomings.

DR. SLATER: It appears that any individual working in this field can standardize his results with his own apparatus. However, it has been demonstrated that photometric analysis of one paper electrophoretic pattern by different machines has been productive of different results

MISS von FRIJTAG DRABBE: You are certainly right.

DR SLATER· I think that in accepting paper electrophoretic data one has to be careful and know the author's reliability. Paper electrophoresis is becoming widely used and in many instances by individuals not well versed in the fundamentals of electrophoresis

MISS von FRIJTAG DRABBE. I do think that every hospital that would be using paper electrophoresis should establish its own normal values, with its own equipment, and then use that as a base to go by, and not work with some other author's figures, so to speak

DR. SLATER I think that one of the main advantages of paper electrophoresis is its use as a screening method Even without analyzing the protein components quantitatively, it is possible to determine rapidly qualitative and semi-qualitative changes simply by gross inspection of the patterns. This method is to protein analysis what paper chromatography is to amino acid analysis.

DR GITLIN· I would agree, we deal with much smaller levels, but with reference to the clinical syndrome of agammaglobulinemia there are many different states in which you won't find the gamma globulin on a pattern of any form.

MISS von FRIJTAG DRABBE· I do agree with you that the clinical findings and the patterns should definitely go hand in hand. The diagnosis should not be based on the findings in the pattern only, definitely not.

DR GITLIN But if you do the gamma globulin quantitatively, you can determine whether the patient has agammaglobulinemia without knowing the clinical history.

MISS von FRIJTAG DRABBE· How could you think that the gamma globulin could be determined with greater accuracy? What would you suggest as a screening method for that?

DR GITLIN Well, there is a rapid screening method. If you have a patient who is not type AB and who is not between the ages of four and twelve weeks, if you test for isohemaglutinins and they are absent, you have an 80% chance that the patient is a true agammaglobulinemia Using that as a screening method, we then go on to immuno-chemical procedures and determine the gamma globulin in that way.

MISS von FRIJTAG DRABBE· But again, that has limitations, too.

DR. GITLIN· I agree it has limitations; the error is very large. But then again, if it is 25 mg.% or below the patient definitely has agammaglobulinemia.

MISS von FRIJTAG DRABBE: Well, of course, as far as the paper patterns go, this is the only experience I have on the agammaglobulinemia. The pediatrician was suspecting it, and asked for the pattern, and he has been treating the child as a case of agammaglobulinemia, and the child is well now as long as he is being treated with gamma globulin, so that is what the diagnosis is based on.

DR. SLATER: I wonder if you think the use of a normal serum used as a standard of reference with each set of separations would be better than attempting comparisons with free electrophoresis. This might be especially true in diseased states. Free electrophoresis methods measure all components of sera, lipids and carbohydrates, as well as proteins On the paper one is staining rather specifically the proteins.

MISS von FRIJTAG DRABBE: I think that by all means, if one works with paper, that the paper pattern should be used as a standard reference, naturally. The Tiselius having its limitations not only in precision, but also its limitations, as you pointed out, by indicating the carbohydrate protein and lipid proteins. You would have to use special staining methods if you want to indicate these substances on the paper. They are there, but you don't see them, if you stain with amido Black 10-B. However, if you use specific stains, specific dyes, and dye procedures, you could bring them out. But that has not been worked out fully, as yet.

There is another advantage that I would like to point out in paper electrophoresis, and that is, if you have a strongly turbid sample with a very high lipid content, you cannot do free electrophoresis, but you can easily do a paper pattern, the lipid content doesn't interfere in the least

DR. FOX I am a little confused about the constant indications that there are so many inaccuracies here If you use similar equipment with similar voltage and make the measurements the same way, shouldn't two people in two different places agree on the same sample? Isn't the principle the same? Why should there be this difference? Why should one get trailing in one case, and not in another?

MISS von FRIJTAG DRABBE: Everybody should get some trailing, I mean that has been found already by several authors. When we first started out working with paper, we didn't notice it We work now with three chambers. Well, the three chambers will give identical patterns if, say, there is a myeloma, you will get a myeloma pattern in each chamber However, there will be variations in the values of the individual fractions which are due to slight differences, probably in voltage, in electro-endosmotic flow, in the temperature constancy in the refrigerator, and things like that. The precision for our three chambers is slightly different, but the gross overall picture is the same For instance, we have made comparisons between the box that was made by Arthur H. Thomas that I showed you. We made 10 runs at 250 volts, 5 to 6 hours, ionic strength 0 05, temperature 5 degrees. We worked with Grassman 17 hours, 100 volts, temperature 10 degrees and ionic strength 0 1. The patterns are quite comparable.

DR. SLATER It has been our experience that careful standardization of voltage

output is not necessary. The electrical properties of the proteins provide that the electrophoretic separation for any serum will be repoducable. Only approximate control of time and current is necessary; the unit can be shut off when the pattern has separated the desired distance. The added expense of careful voltage controls is unnecessary

MISS von FRIJTAG DRABBE. If we start a run on the Grassman-Hannig, we start out with a voltage of 100 I know that sometimes the voltage drops to maybe 95-98, but I am not concerned with that. It is just a rough value It is the same at 250 volts. It need not be very exact.

DR. FOX. If you take radio-iodinated serum albumin and then chopped your paper up into counts, would all the counts fall in one place, or would they be scattered around, in view of this trailing you describe?

MISS von FRIJTAG DRABBE I didn't exactly follow you

DR. FOX If you took radio-iodinated serum albumin, as one component, and then chopped the paper in pieces, and did counts on each centimeter, would all this activity be in one place, or would it be scattered around? And would you have this trailing, whatever that means?

MISS von FRIJTAG DRABBE I haven't done that, but certainly per centimeter, I don't think you would have exactly the same amount The paper has limitations, too.

DR SLATER In working with radioactive I^{131} labelled albumin one finds trailing of the radioactivity in the path of migration of the albumin This occurs in proportion to the amount of albumin bound to the paper.

MISS von FRIJTAG DRABBE But the paper isn't completely homogeneous.

MEMBER Are different media, different papers being investigated? I suppose they are

MISS von FRIJTAG DRABBE: Yes, different papers have been used. We work with Whatman No 1 For techniques where you use electromigration between glass plates a much heavier paper is used. We have tested several papers but we have returned to Whatman No 1 as being, for our purposes, the most satisfactory It has on one side a smooth surface, and on the other side a slightly rougher surface, the proteins partly travel through the paper, and partly travel on the surface of the paper. We have always a more strongly colored pattern on the top than on the bottom of the paper You must at any rate use a paper that hasn't too much of a special grain in it. We once worked with a paper that had a very faint ridging in it. You should have seen the patterns.

DR SLATER· So far there have been no reports of paper which will not adsorb proteins?

MISS von FRIJTAG DRABBE Not that I know of.

DR. SLATER: H.A. Jermyn reported in Nature, Oct. 17, 1953, a method of altering a paper by chemical treatment. This involved coupling hydrazine derivatives to the aldehyde groups of the cellulose. He claimed that a considerable decrease in trailing was produced. This form of treatment may be well worthwhile applying in this clinical work.

DR. FOX: How much is the isotope spread out beyond the spot in your experiment?

DR SLATER: The paper adsorbs protein no matter what the concentration of the component being analyzed If a small concentration is being analyzed the entire amount may be consumed by the paper in the path of migration before it reaches the point it should be at, based on its mobility characteristics. In our studies of iodinated albumin, sufficient concentrations were used so that only a small fraction was lost due to adsorption to the paper.

MEMBER· With a given paper, though, this is a fairly constant amount in comparison with the total amount of albumin.

MISS von FRIJTAG DRABBE Fairly.

DR SLATER: Since albumin has a strong binding capacity and is the first component to traverse the paper during a separation it probably saturates the paper so that the components following behind are not adsorbed.

MISS von FRIJTAG DRABBE: It is a wonderful thing to theorize about. Someone asked me about the technique of applying the sample to the paper We pre-treat the paper by inserting it into the chamber, starting the current, and leaving it connected about two hours before we apply the serum. Then when it is time to apply the serum, I take a paper strip of 1 1/2 to 2 mm. width and 1 1/2 in. long, dip that into the serum, tip off the excess, and drop the paper strip carefully on the pencil line I use a wider strip if I have a serum with a low total protein, and a strip narrower than 1 1/2 mm. if the serum contains a large amount of protein We seem to get lower gamma globulin values this way It is possible that by using this method we get, for some obscure reason, less absorption of albumin by the paper than when we actually apply the serum to the paper by moving a pipette back and forth.

MEMBER: I would like to ask a question in connection with this trailing effect. When you added a certain small increment you got a large amount of globulin appearing and no matter how much more you added, you always had the same increment. Well, you get the same effect with the moving boundary. I think it might be due to interaction. Is there an interaction there?

MISS von FRIJTAG DRABBE: All we can say with certainty is that there is some interaction between albumin and gamma globulin.

MEMBER· So that the globulin carries albumin along with it.

MEMBER: Presumably it shows up in the descending slope of the albumin.

MEMBER: You never really get a good gamma globulin peak in the patient with agammablobulinemia after treatment with gammaglobulin although here you get a good globulin peak with gamma globulin added in vitro.

MISS von FRIJTAG DRABBE: When we added large amounts of gamma globulin, yes.

DR KRETCHMER· Yes, and when she added 10 gamma she got 60 back; and when she added 50, she got 100 back -- that 50, I think, may well be the patient's own globulin which is now not trailing, but is being added as an increment to the material you have added

DR SLATER: I think there is a simple answer to all of this, and that is that this method just is not sensitive enough One cannot draw conclusions about agammaglobulinemia by this technique although evaluation of greatly diminished concentration is possible The method is likely not sufficiently sensitive for a final diagnosis of agammaglobulinemia

MISS von FRIJTAG DRABBE That's right

DR. PRESSMAN By using an additional amount of gamma globulin you may be able to pick up an increment.

MISS von FRIJTAG DRABBE. Yes, that could be However, I also feel that we should have far more values in different cases and many more experiments than I have

DR. PRESSMAN. I'd like to ask, what was that black?

MISS von FRIJTAG DRABBE· Amido Black.

DR PRESSMAN Are you dyeing all different proteins to the same degree?

MISS von FRIJTAG DRABBE· Well, that again is a relative thing. It is more or less so.

DR PRESSMAN Would it be a factor of two, or would it be a correction of 5 or 10% for different proteins?

MISS von FRIJTAG DRABBE. We have some evidence that albumin stains to a slightly greater extent than does gamma globulin, but the difference is small.

DR PRESSMAN I think it is a remarkably slight difference

MISS von FRIJTAG DRABBE Yes, but you see you can change that factor. What I have not pointed out is that after the run is completed, the next morning these patterns are dried for 10 minutes at 100°, thereby deliberately denaturing, or partially deteriorating the proteins so that they become more susceptible to the dye, which results in a better dye uptake So the color is much lighter if you dry your pattern in air, then when drying it in an oven

DR. KRETCHMER: Actually, you heat-denature the protein before applying the stain

MISS von FRIJTAG DRABBE: Yes, that's right.

DR. PRESSMAN: Then this explains why the albumin takes up as little as it does. Albumin usually has a high binding capacity when compared with other proteins and by denaturing the albumin, you lower its binding capacity to where it is similar to other proteins?

MISS von FRIJTAG DRABBE: That is quite possible. Now of course we have not worked with the other fractions; we have only worked out the ratio of dye uptake for gamma globulin and for albumin, because I didn't have the other purified globulins available.

DR. PRESSMAN: I think that even if you stain without drying the denatured albumin, you get this same relationship with the same dye uptake by albumin as by gamma globulin. First of all, you are troubled about the solubility of the dye, you have to render your dye insoluble, and albumin is known to bind simple substances and simple dyes to a very great degree, whereas globulin does not. And if you carry that over, I think you can generalize for at least most negatively charged dyes, that the albumin is going to bind rather strongly.

MEMBER: Except that for some reason or other it binds with apparently the same ratio as the other proteins.

MEMBER: Do you think albumin will bind the same as other proteins?

MEMBER: No. Usually albumin dye binds more. This amido-Black apparently is some exception I haven't worked with it but I know that everybody that has reported on it got exactly the same results.

MISS von FRIJTAG DRABBE What we now base our conclusions on is the comparison with the Tiselius in general, and taking into account the Beta anomaly it is so good that there seems to be an approximately or almost equal affinity for the dye of all the protein fractions.

DR. EDER: Some of the wave fronts are not perpendicular to the direction of migration but lag at the sides. Does this not cause error in analysis of the curves?

MISS von FRIJTAG DRABBE: Yes, but only if there is a marked curvature. You may find in the albumin a slight asymmetry. For instance if you take the paper out of the chamber to put it into the oven and let some time elapse between taking it out and putting it into the oven, the buffer here at the top of the bridge flows somewhat toward the center of the paper where it evaporates, and you get a compression of the pattern which may result in an asymmetry in the albumin peak. So you will find a steep slope on the forward side, and you may find some of it flowing out to the right-hand side although it is a homogeneous fraction. This effect may be prevented by blotting the

paper where it rests on the bridge after removal from the chamber.

MEMBER: Do I understand you correctly that the color resulting is blue?

MISS von FRIJTAG DRABBE: Yes, a beautiful blue.

MEMBER: Why don't you work with color filters and cut out much of the intervening colors from the paper it is on?

MISS von FRIJTAG DRABBE· We could, and it would increase the sensitivity.

DR FOX: Would the chemical composition of the dye amido Black 10-B help explain the tendency of the albumin to bind?

DR PRESSMAN· I think I can safely say that the chemical construction certainly has a lot to do with it.

MISS von FRIJTAG DRABBE One thing that we discovered is that if we added bromphenol blue to serum before making a run, that if we afterwards stained the pattern with amido Black 10-B that the bromphenol blue somehow got lost. I mean there was apparently a greater affinity for amido-Black 10-B, and that binding was stronger than that of the bromphenol blue to the albumin Up to the moment of dyeing, after the pattern came out of the oven, you could still see a clear blue line where the albumin was But afterwards, in comparing patterns to which bromphenol blue had been added, with those to which no bromphenol blue had been added, the albumin values came out exactly the same

CHAIRMAN RAPOPORT· The next presentation, by Dr. McCrory, is "The Application of Paper Electrophoresis to Animal and Human Material"

DR McCRORY· The mechanism of proteinuria in nephrosis is a subject of fundamental importance. We have undertaken the study, by paper electrophoresis, of proteins in serum and urine in children with nephrosis to obtain estimates of the concentration of the various separate protein fractions, albumin A_1 and A_2 globulins, B-lipoprotein and gamma globulin

If we can obtain such information we can then calculate the separate protein excretion rates as clearances Such measurements would possess physiological value in terms of the mechanism of proteinuria. Much of the work I will discuss here is preliminary and deals mainly with methodology

We have arbitrarily assigned numbers to the five major components of serum proteins appearing as peaks on paper patterns Thus, albumin is I; A_1-globulin, II; A_2-globulin, III; B-lipoprotein, IV; and gamma globulin, V The identification of the various fractions obtained on paper patterns has been based on the similarity of the quantitative values of dye staining of the bands on patterns to the classical Tiselius,

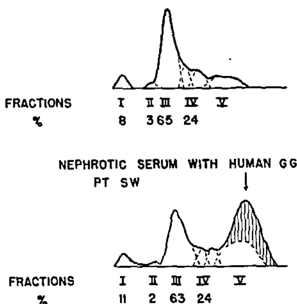
Mobility calculations can be made to identify separate fractions but they require rigid controls of the many variables, (endosmotic flow, temperature, type of paper, etc.), inherent in paper electrophoresis.

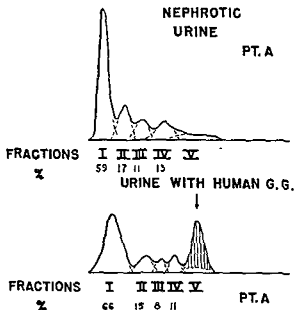
Another approach to this problem is by admixture of pure fractions. This method indicates identification by the superimposition of the added known pure protein peak in reference to the specimen in question. We can obtain excellent separation by paper electrophoresis of such pure fractions when mixed in vitro. We studied a mixture of human crystalline albumin, metal combining beta-lipoprotein and gamma globuli (Fig. 25). The artificial mixture gave distince peaks at I, IV and V, as expected with no significant protein at II and III. In Fig. 26, the area designated as V is shown with and without added human gamma globulin The position of added human gamma globulin in the nephrotic serum is seen to be limited to the area designated V, with no distortion apparent in the composition of the other protein fractions. In Fig. 27, a similar result is observed when human gamma globulin was added to urine, attesting to the reliability of the designation of V peak as gamma globulin.

The study of fluids low in protein concentration was possible only after concentration by dialysis We prepared such specimens (urine and ascitic fluid) by dialyzing the material in cellophane in 20% Dextran in buffer at 0°C for 18-24 hours. Fig. 28 shows a pattern obtained by direct application of untreated urine as well as the same urine after dialysis. No distortion of the pattern was apparent, in fact separation appeared to be improved by dialysis and concentration. In Fig. 29 the pattern of protein in ascitic fluid is presented with the pattern of the same subject's serum proteins The protein concentration of the ascitic fluid prior to dialysis was 56 mgm % We feel this method is quite suitable for studies of the various protein fractions in serum, urine and ascitic fluid

The albumin/globulin ratio (A/G Ratio) of urine has long been a matter of interest. It has been claimed that a high urine protein A/G ratio, meaning a preponderance of albumin in the urine protein, is commonly found in patients with nephrosis while patients with nephritis have urine protein A/G ratios of small magnitudes. If a distinction could be made between these diseases by this measurement early in the course of the disease it would be of immeasurable value. We have calculated urinary A/G ratios on a number of our children. We have arbitrarily divided them by clinical findings into two groups. One group - "Nephrotic" - being used to include those children who demonstrate complete remission with disappearance of proteinuria and diuresis by steroid therapy; the other group - "Nephritic" - being used to include children failing to show complete remission and having associated findings of (1) elevated NPN (2) hypertension and (3) hematuria. No significant difference in A/G ratios was apparent. This could mean that either we do not have a true group separation or that such a difference is not apparent.

We do not have measurements of glomerular filtration rates on enough of the children to be able to make any comment about them. Changes in proteinuria in patients can be associated with changes in the urinary A/G ratio. Urine A/G ratios done on two children before and after they went into complete remission revealed that after urine protein had decreased (from 3.5-4 gms. in 24 hours, down to 250 mg. in 24 hours), the A/G ratio decreased (2-1 8 to .8 and .6 respectively). This low ratio is approaching





Figs. 26 & 27: Effect on electrophoretic pattern of addition of human gamma globulin to nephrotic serum and urine.

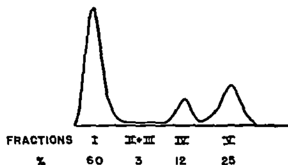


Fig. 25: In vitro mixture of pure plasma protein fractions (albumin, metal-combining Beta lipoprotein, and gamma globulin).

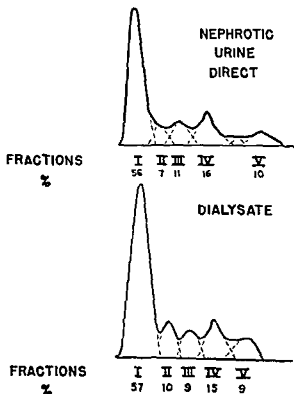
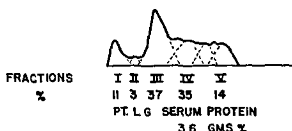


Fig. 28: Effect of concentration by dialysis on urine protein pattern



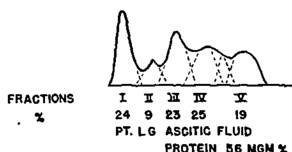


Fig. 29· Comparison of concentrated (dialysis) ascitic fluid proteins with serum protein pattern

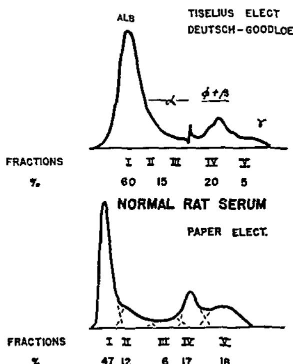


Fig 30 Comparison of electrophoretic patterns of rat serum obtained by Tiselius moving boundary and paper strip

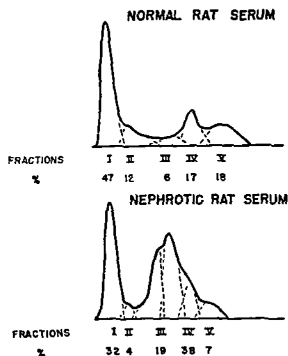


Fig. 31: Comparison of normal and nephrotic (Heymann) rat sera.

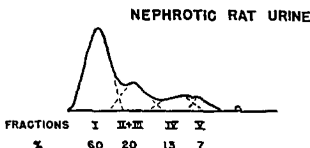


Fig. 32: Urinary proteins of nephrotic rat Compare with Fig. 28.

the figure for urine A/G ratios of 0.51 of normals of Rigas, D.A. and Heller, C.G. (J.C.I. 30·853, 1951) These changes in the A/G ratio in the same patients occurring in association with diminution in total proteinuria clearly indicate that the quantitative aspects of proteinuria are important in assessing the meaning of the urinary A/G ratio.

MEMBER: Do the absolute values stay the same for the globulins while the albumin decreases?

DR. McCRORY· No, all of the fractions will decrease absolutely, but the relative amount (i.e. percentage) will, in effect, increase for globulins, since the major component albumin is the most markedly decreased. Leaving the problem of the significance of urinary A/G ratios still unsettled I would like to show you data on protein clearances. It is our aim to relate the clearances of the various serum proteins to glomerular filtration rate and clinical course of the disease. This approach may provide information of value in understanding the mechanism of renal excretion of protein . . We have results on protein clearances only on a small number of children to date The clearances are calculated from timed 24 hour urine collections. Total protein in serum and urine was measured by the Biuret method, fractions estimated by paper electrophoresis The results in 5 children are shown in Table 2.

Table 2

SUBJECT	SERUM ALBUMIN GM %	PROTEIN CLEARANCES ML/MIN C_{AL}	C_{AG}	C_{GG}	SERUM GAMMA-GLOB. GM%	URINE PROTEIN GMS/ 24 HRS.
P	7	4	16	04	.4	4.1
F	5	34	18	.01	58	3.0
LF	41	.47	27	01	.19	4.4
W	23	84	.33	.08	.45	5.0
G	.22	81	34	.04	.25	1.9

1 - C_{AL} - Albumin clearance

2 - C_{AG} - A_1 Globulin clearance

3 - C_{GG} - Gamma globulin clearance

The serum albumins levels and the total urinary protein excretion are included. The inverse relationship of albumin clearance to serum concentration is obvious The alpha-1 globulin clearance also is seen to increase with the albumin clearances. The gamma globulin clearance, while extremely minute, did not change in relation to the albumin clearance or to serum concentration of gamma globulin These results would support the concept that a major factor in renal loss of protein is an abnormally permeable membrane resulting in increased filtration of smaller molecular weight serum proteins in abundance. The albumin molecule is the smallest of the serum

proteins and its clearance is highest. We hope to obtain similar data for all fractions. The low gamma globulin values may be due to the method in part. We are aware of the necessity for standardizing the paper electrophoresis technique if we are to use this method for quantitative study of proteins.

In addition to the work on humans we have also studied the plasma protein patterns in rats with anti-kidney nephritis. Dr. Walter Heymann has provided us with specimens from his animals. The pattern of serum proteins in the normal rat obtained by paper electrophoresis was practically identical with that found by Tiselius technique by Deutsch, H.F. and Goodloe, M.B (J B.C. 161.1, 1945) (Fig. 30) The peak at the site of the alpha-1 globulins was small and no peak was seen where alpha-2 globulins would be expected. The pattern of serum proteins in the nephrotic rat (Fig. 31) were very similar to the pattern in the human with nephrosis. A great increase was seen in the area of the alpha-2 globulins. The albumin was not as low in the nephrotic rats as in the nephrotic humans. We do not have total protein values for these specimens but Dr. Heymann tells me they were about 3 grams % The composition of urinary protein was also similar in the nephrotic rats to that seen in the human (Fig. 32). The major components were albumin and alpha globulin Dr Heymann has been interested *in the urinary A/G ratios of the nephrotic rats and I can provide the data for four* animals studied to date. Two had low urinary A/G ratios (1.7 and 1.3) and both had elevated NPNs and hypertension, duration of disease was 3 months and 13 months. The two others had high A/G ratios of urine, 3.2 and 4 0. Both of these animals had proteinuria and no elevated NPN, though blood pressure was elevated in one. The data are interesting but quite inadequate for any answer to this problem

DR. FOX Did these rats have edema?

DR. McCRORY Walter would have to answer that

DR. METCOFF: Some years ago we were interested in protein deficiency in rats(1) and ran quite a few electrophoretic patterns on their plasmas. I believe the patterns were similar to the ones you observed in the nephrotic rats. The changes in total circulating amounts of the various plasma components were determined. The effect of diet protein deficiency on reducing circulating albumin and increasing the lipoproteins was striking. The rats were not nephrotic.

DR. BARNETT: May I ask how Blackman and Davis measured the proteins in the urine?

DR. McCRORY. Salt fractionation.

MEMBER: What changes occur in serum proteins in the patient following diuresis?

DR McCRORY. The pattern can show all degrees of change including complete return to normal for all fractions The degree of change correlates in general with the degree of diminution of urinary protein excretion The patients showing complete

(1) Metcoff, J., Darling, D.B., Scanlon, M.H , and Stare, F.J., J. Lab. Clin. Med. 33:47, 1948

remission including disappearance of proteinuria may have normal serum protein patterns after diuresis. All degrees of change from the abnormal pattern of the nephrotic to the normal can be encountered.

DR METCOFF· This is in accord with some unpublished observations of S. H. Armstrong Jr. and C. A Janeway since following diuresis, in this instance measles, there was a periodic improvement in the serum pattern toward the normal, and a simultaneous inverse decrease in the urine protein. Does gamma globulin reappear in serum upon diuresis, before other changes are present?

DR GITLIN· I believe the question cannot be answered by this method in view of the limitations of the method for quantitative changes involving small amounts of gamma globulin

DR. McCRORY: We found that the albumin, alpha-1 globulin and gamma globulin fractions usually rose with diuresis while the alpha-2 globulins and beta-lipoproteins decreased to normal. The question of relationships between serum and urine gamma globulin is as yet unclear. We did not find a close relationship (as shown in Table 1) between level of serum gamma globulin and amount in urine This would suggest that urinary loss of gamma globulin is not the major determinant of the serum concentration

MEMBER Doesn't gamma globulin rise after diuresis? We found this to be true in a few cases we studied

DR McCRORY: If the proteinuria diminishes or disappears with diuresis, the total serum proteins including gamma globulin increase. If no pronounced decrease in proteinuria is associated with diuresis we have not found consistent change in the level of serum gamma globulin The direction of change seems to be associated with the effect on proteinuria rather than diuresis alone.

DR HEYMANN· Some years ago Blackman (2) published that a high globulin excretion in the urine seemed to be associated with a progressive renal disease in patients suffering from the nephrotic syndrome Over 35% of globulin in the urine seemed to be a sign of poor prognosis He used the ammonium sulfate method, which [illegible] is work stimu- [illegible] Dr. Pillemer, [illegible] -en in different phases of their disease were analyzed in thirty-six different determinations. Three of these twenty-one children had the nephrotic syndrome in its malignant progressive form Two of these children have died of renal failure; one is still alive, but has a fixed specific gravity, has azotemia and has had convulsions repeatedly. Four determinations were obtained in these three children. The albumin-globulin ratio in their urine yielded an average figure of 1.05 The globulin excreted in their urine amounted to 48% of the total proteins lost in the urine Twelve of the twenty-one patients *examined*

(2) Blackman, S S , Goodwin, W E and Buell, M V. On the relation between the concentration of total protein and globulin in the urine and the pathogenesis of certain renal lesions in Bright's disease. Bulletin Johns Hopkins Hospital 69:397, 1941.

have had their disease long enough to permit us to believe that their disease is taking a benign course. In average figures the albumin:globulin ratio in their urine was 2.1 and the globulin amounted to 33.6% of the proteinuria. In two children we do not know as yet how the course of the disease will be. The prognosis is in doubt; the albumin:globulin ratio in their urine was 1.8, and the globulin excretion amounted to 37%. The material is too small to permit any definite conclusion, but tentatively it would look as if a globulin excretion of more than 45% and an albumin:globulin ratio of less than 1.5 would be a bad prognostic implication Among the children examined, there were, however, already two exceptions. One child whose disease took a benign course had a 44.9% globulin loss in the urine; the other child 47% These exceptions should make us very cautious in evaluating any single result. As a whole, it seems as if this whole problem deserved further investigation as the original Blackman contention may be substantiated to a certain extent. It was also found that the nephrotic hematuria is not necessarily, accompanied by a greater loss of globulins in the urine or by a low albumin:globulin ratio Three children were also followed in this respect during the course of ACTH treatment. In two of these children the albumin:globulin ratio increased and the globulin excretion decreased correspondingly; in the third child this did not happen. Further results have to be obtained before any conclusion should be reached.

DR. BARNETT: Walter, how did these ratios correlate with other indications of their disease; and, secondly, did you see changes in these ratios in the same child at different stages o the disease?

DR HEYMANN: In one and the same patient, the albumin:globulin ratios in the urine were usually quite constant, but again, there were a few exceptions to this observation, in that two or three children did show quite a fluctuation of their values at different times.

DR. McCRORY: *The results of our studies to date on urinary A/G ratios seem* to suggest that one finds a predominance of albumin in proteinuria above 1-2 grams per cent and an A/G ratio of 1 or greater regardless of the presence or absence of signs of nephritis As protein excretion falls below this level, whether in remission or because of renal insufficiency, we find the A/G ratio decreasing. This would suggest that A/G ratios have limited prognostic value They apparently may vary with changes in proteinuria in the same patient

DR. HEYMANN: The albumin:globulin ratio is, according to our findings, quite independent of the intensity of the proteinuria

DR. McCRORY: Do you have ratios of less than 1 with degrees of proteinuria of 3 and 4 gms.?

DR. HEYMANN: Yes we do. In one and the same patient the albumin:globulin ratio is usually quite constant. Again, however, there are exceptions to this: One child for instance, had four different examinations; three yielded ratios between 2.5 and 3.0, while once a ratio of 1.48 was obtained in between. The cause of the disease in that particular patient was and still is benign.

DR. LAUSON: It seems to me that if you have data on the individual protein fractions it is unnecessary, and misleading, to speak of A/G ratios. The "G", after all, is made up of large and small globulins, for which glomerular permeability varies greatly. I would like to enter a plea that the urinary excretion of individual proteins always be considered in relation to the corresponding concentration in the plasma. No matter how the proteins get through the glomerular walls, through pores or by diffusion, other things being equal, the rate of transfer ought to be more or less proportional to the plasma concentration of the individual proteins.

In the free electrophoretic data (see Table 3), the ratios of gamma globulin/albumin clearances were often high. Similarly, the ratios of alpha-1 globulin/albumin are quite large. I believe also that the clearance of thyroxin-bound globulin is a large fraction of the concurrent albumin clearance in patients with the nephrotic syndrome.

It is possible to achieve some unification concerning the passage of various plasma proteins into the urine. Following the lead of Malmros and Blix (Malmros, H. and Blix, G., The plasma proteins in cases with high erythrocyte sedimentation rate. Acta Med. Scand., 1946, Suppl. 170, 280). Chinard, Eder and I (unpublished) assembled the data then available in which free electrophoretic analyses of simultaneous urine and plasma specimens were made in several types of renal disease. By assigning the value of 100 per cent to the ratio: Urinary albumin concentration/plasma albumin concentration, the U/P ratios for the various globulins can be expressed as a percentage of the concurrent albumin U/P ratio. Since urine flow is the same for all of the proteins, this calculation expresses the relationship of the various globulin clearances to that of albumin. These data are summarized in the accompanying Table.

Table 3

Summary of renal clearance of globulins relative to that of albumin in several types of renal disease.

(All analyses were done by free electrophoresis)

Globulin clearance/Albumin clearance x 100
(range of observations)

	α_1	α_2	Total α	β	γ
Nephrotic syndrome [2,3,4,5,6]	17-79	4	1-12	3-8	6-69
Chronic and terminal glomerulonephritis [2,4]	-	-	21-48	30-50	42-73
Diabetic glomerulosclerosis [7]	58-103	13-25	-	12-24	19-31

References:

1. Chinard, F.P., Lauson, H.D., and Eder, H.A.: Unpublished.
2. Luetscher, J.A., Jr.: Electrophoretic analysis of plasma and urinary proteins. J. Clin. Invest., 1940, 19, 313. (Cases I, II, and IV) (V and VII).
3. Longsworth, L.G., and MacInnes, D.A.: An electrophoretic study of nephrotic sera and urines. J. Exptl. Med., 1940, 71, 77. (Cases B.B., S.G.)
4. Malmros, H., and Blix, G.: The plasma proteins in cases with high erythrocyte sedimentation rate. Acta Med. Scand., 1946, Suppl. 170, 280. (Case 999, 1344 and 1408).
5. Routh, J.I., Knapp, E.L., and Kobayashi, C.K.: Electrophoretic studies of plasma and urinary proteins in children with lipoid nephrosis. J. Pediat., 1948, 3, 688. (Cases D.M., and H.A.)
6. Luetscher, J.A., Jr.: The effect of a single injection of concentrated human serum albumin on circulating proteins and proteinuria in nephrosis. J. Clin. Invest., 1944, 23, 365. (Cases I and II)
7. Rifkin, H., and Peterman, M.L.: Serum and urinary proteins in diabetic glomerulosclerosis Results of electrophoretic analysis. Diabetes, 1952, 1, 28. (Cases 1 - 3)

CHAIRMAN RAPOPORT: Dr. Slater, do you have some additional material on that?

DR. SLATER: I thought it would be interesting to consider some of the aspects of protein metabolism in the nephrotic patient with special reference to the electrophoretic components of the serum and urine. In Fig. 33 there appear five sets of patterns, comparing the serum and urinary proteins from five patients with nephrosis. The upper pattern in each bracketed area is of the serum and the lower pattern of urine. It is apparent that in the upper two patterns the main components of the serum are clearly recognized, although some distortion of the α_2- and b-globulin fractions exists. The serum patterns in the lower three instances are grossly deranged and exhibit markedly decreased components and a greatly increased fraction in the α_2-region which is chiefly lipid containing material.

Just as the serum patterns are different from one to another patient, so also are the corresponding urinary protein patterns. In every patient studied the chief urinary constituent was albumin. Likewise there appeared consistently a b-globulin component. The presence of other components in the urinary protein patterns was quite variable from patient to patient. In the α_1-α_2-globulin region variable amounts of three components were seen, the most rapid usually appearing in the greatest concentration. These do not correspond to the main electrophoretic components of the corresponding nephrotic serum or normal serum. Patient 3 had material of mobility slower than γ-globulin. My point in demonstrating these differences is simply this: in the nephrotic state there may be produced either abnormal proteins or greater or lesser quantities of normal serum proteins or both. The appearance of some components in the urine which may not have been identified in the serum of normal or nephrotic patients provides the possibility of further characterization of these proteins.

DR. GITLIN: Dr. Slater, before you continue, I think something ought to be pointed out, you are calling these alpha, beta, and gamma globulins. We now have seven different alpha-1 acid glyco-proteins of very small molecular weight, so that these smaller molecules might conceivably have increased clearances relative to other alpha, beta or gamma globulins that are present in the serum.

DR. SLATER: The nomenclature applied to these proteins has been simply on the basis of electrophoretic mobility of the component referable to the normal serum pattern. It has nothing to do with the actual identity of specific proteins in any one component.

Separation of urinary proteins by means of zone electrophoresis in a starch supporting medium was carried out in order to provide larger quantities of material for further analysis (see Zone Electrophoresis in a Starch Supporting Medium, H. G. Kunkel and R. J. Slater, Proc. Soc. Exp. Biol. Med., 80, 42, 1952.).

Fig. 34 illustrates a typical pattern. The abscissa represents the 1 cm. starch block sections which were eluted in saline and the ordinate represents the blue color derived when Folin-Ciocalteau (Electrophoresis of Proteins on Filter Paper, H.G. Kunkel and Arne Tiselius, J. Gen. Physiol., 35, 1, 1951), analyses were carried out on each eluate. Three main components are noted, the albumin at tube 40, an a-globulin

components of rapid mobility at tube 32 and a b-globulin component at tube 15.

Ultracentrifugal analyses of these three peaks was carried out in order to define the number of constituents in each and also relate them if possible to known constituents of normal serums. The albumin component had an S_{20}=4.4. Since this is known to be identical immunologically to normal serum albumin it is assigned a molecular weight of 62,000. The b-globulin component had an S_{20} =approx. 5.1. The iron binding capacity of this component was found to be high. This is in agreement with the findings of Smith, Shulman and Morgenthau, (Advances in Pediatrics, Vol 5, p. 195, 1952, Year Book Publishers, Chicago) that urinary protein in patients with nephrosis has a high iron binding capacity. Since this component migrated as one peak in the ultracentrifuge it seemed reasonable that a large proportion of this component was made up of b-globulin metal-combining protein. On the basis of this finding and the S_{20} =5.1 a molecular weight of 90,000 was assigned.

The large a-globulin component had a S_{20} =3.1 and on this basis was suggestive of a molecular weight of approximately 45,000. Despite the presence of greatly increased concentrations of high molecular weight apoprotein components in the serum, none were identified in these urinary protein fractions. γ-globulins were of the largest components (MW approx. 150,000). The findings indicate that only smaller molecular weight proteins are passed through the damaged kidney. This work, of course, is being reported in a preliminary stage. Further studies are being carried out on the fractionation and identification of urinary protein constituents.

MEMBER: Excuse me; do you have that same patient's serum, by any chance? Were you able to prove that there was such a 45,000 fraction in the serum?

DR. SLATER: The studies have not progressed sufficiently to compare all of these fractions in the urine with those of the serum. Some of the urinary components had no recognizable counterpart in the serum. Possibly immunological identification of serum-urine counterparts would be a feasible approach to the problem.

DR. GITLIN: Dr. Slater, because you brought up the question of abnorman proteins, one should note that these are not necessaryly abnormal proteins. The reson you see a beta peak or a beta spot on your electrophoresis without seeing the alpha spot when compared to the urine, is because you have a lot of other beta proteins in there. If the concentration of the serum fraction were increased you would show that this alpha-1 peak present in the urine is also present in the plasma.

DR. SLATER: It would certainly be present in plasma unless of course it is excreted by the tubule or urinary tract. Assuming that it comes from the blood it should be demonstrable, if only by immunological means. If the component has a specific biological activity, measurements could be carried out by that yardstick.

DR. EDER: Can I show a table at this point in reference to the proteins that Dr. Slater mentioned?

We have studied the effect of maintenance cortisone therapy on plasma proteins and lipoproteins. In Fig. 35 are shown the data from such a study. During the period

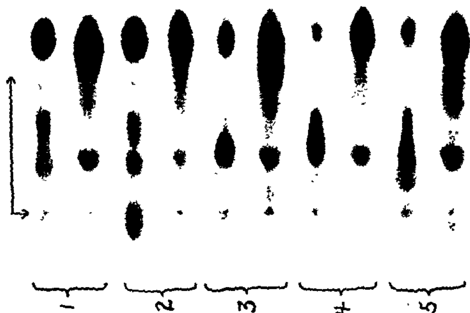


Fig 33 Serum (upper) and corresponding urine (lower) protein patterns from five cases of *nephrosis* separated simultaneously on one sheet of filter paper

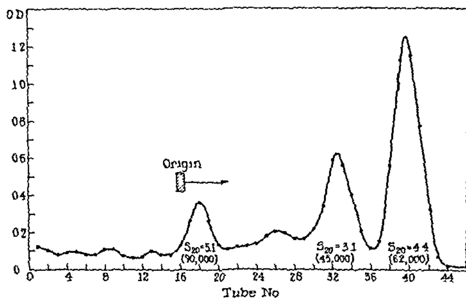


Fig 34 Starch zone electrophoresis pattern of urinary proteins from patient with nephrosis

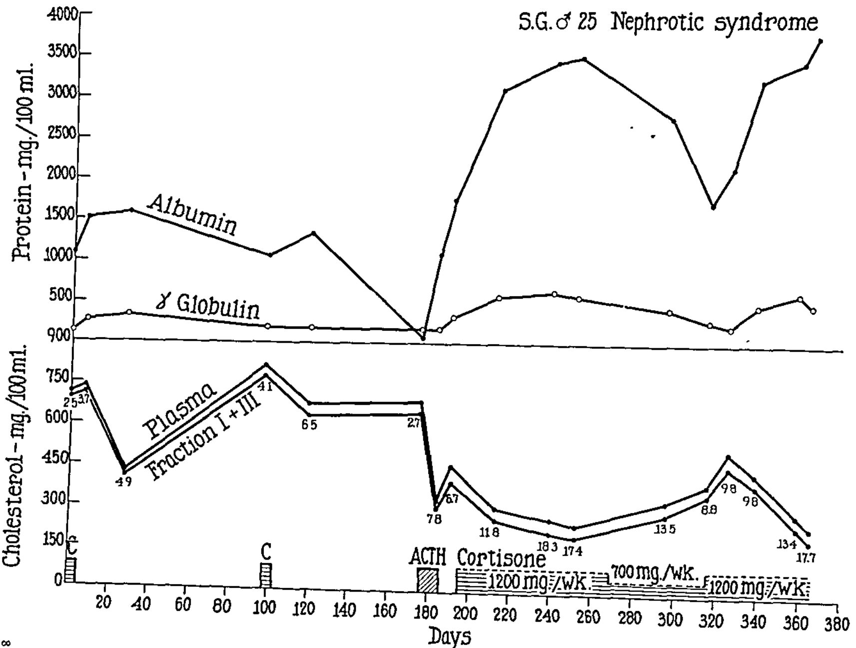


Fig 35: Effect of cortisone maintenance therapy on plasma protein fractions in a patient with the nephrotic syndrome.

of study subject S.G., a 25 year old male with the nephrotic syndrome, received 2 seven-day courses of cortisone. Subsequently he was treated with ACTH for 10 days and this was followed by cortisone in dosage of 400 mg. for 3 days out of every week for 75 days. This was then decreased to 400 mg. four days out of every ten days for 47 days and he was then returned to the original schedule. The proteins were separated by Cohn fractionation. Fraction IV+V+VI which is largely albumin is labelled albumin. Fraction II which is largely γ globulin is so labeled. In the lower section the total plasma cholesterol is plotted and below that is the cholesterol in fraction I+III which is the cholesterol of beta lipoprotein. The difference between the plasma and fraction I+III is equal to the alpha lipoprotein cholesterol. On the maintenance regimen the albumin and globulin rose to normal, the cholesterol decreased by virtue of a drop in beta lipoprotein cholesterol, and the alpha lipoprotein increased Normally the cholesterol in alpha lipoprotein is about 25% of the total. In this patient before treatment, it was 2 7% of the total, and on treatment it rose to 18.3% When the maintenance dose was decreased, the protein and cholesterol values started to return to pretreatment levels. On returning to the original dose this was reversed

The good correlation between the plasma lipid abnormality and the concentration of plasma albumin is especially interesting in view of the studies of Gordon et al. (Proc. Soc Exp Biol and Med , 84:168, 1953) on the role of albumin in the heparin induced clearing reaction

CHAIRMAN RAPOPORT· Dr Gitlin, suppose you take over at this point

DR GITLIN· Just to review some of the things that we presented before on the distribution of plasma protein in tissues, we found large concentrations of plasma proteins in connective tissues and lesser concentration in the cells. We also found that if you give a plasma protein like albumin, gamma globulin, or fibrinogen intravenously, you get a disappearance curve that suggests that half of the plasma protein leaves the circulation within a matter of a few days.

We also presented information suggesting that the plasma protein that leaves the circulation is present extravascularly as preformed plasma protein, and it appears predominantly in the connective tissues. Well, the reason I wanted to give this as a background is because we have some data on nephrosis and found that we had no comparable data for, say, comparable normal patients and for comparable situations.

If plasma protein is removed from the circulation, after equilibrium plasma protein comes out of the extravascular spaces resulting again in a rise in plasma protein in the blood, suggesting that the plasma protein equilibrium is a true dynamic equilibrium That the capillaries are certainly not impermeable to plasma protein is well known

Now to go back to what we have to present, I would like to show data on the nephrotic syndrome, if I may.

Table 4

The concentration of gamma globulin and albumin found in edema fluid of children with the nephrotic syndrome.

	Concentration of Albumin in				Concentration of γ-Globulin in:			
	Serum	Ascitic Fluid	Edema Fluid	Glomerular Filtrate	Serum	Ascitic Fluid	Edema Fluid	Glomerular Filtrate
Average Concentration (gm/100 ml)	0.35 (9)	0.014 (5)	0.005 (6)	0.020* (8)	0.21 (9)	0.010 (4)	0.002 (5)	0.001* (6)
% Retention in	----	96.0	98.6	94.3	----	95.2	99.0	99.5
Calculated Values at Normal Serum Levels with Same Capillary Permeability (gm/100 ml)	3 5	0 14	0.05	0.20	0.72	0 034	0.007	0.003

* minimum concentration $= \frac{\text{excretion of alb. or gamma-glob. mg/min}}{\text{inulin clearance, cc/min}} \times 100$

Table 4 shows some immunochemical determinations which most of you may have seen at the May meeting in Atlantic City. These columns represent concentrations of albumin and gamma globulin in serum, ascitic fluid, edema fluid, and in the glomerular filtrate. To qualify the last, it represents the minimum concentrations of the particular protein in question in the glomerular filtrate, and is calculated on the basis of excretion of albumin or gamma globulin in milligrams per minute. The latter columns represent nothing more than a duplication of Dr. Chinard's work.

We found that for a certain average serum albumin concentration in 9 patients, .35 gms. per cent, there were 14 mgs per cent albumin in ascitic fluid, 5 mgs. per cent in the edema fluid, and 20 mgs. per cent as the concentration in the glomerular filtrate.

Now, if you wish to consider that these fluids represent capillary filtrate, then it would appear that for every 100 ml. of capillary filtrate, at least 96% of the serum albumin is being retained in the peritoneal circulation, 98.6 in capillaries of the skin, and 94 3% in renal capillaries. If you extrapolate it to a serum concentration of 3.5 gms. per cent, that gives concentrations of 140 mgs. per cent in ascitic fluid, 50 mgs. per cent in edema fluid, and 200 mgs. per cent in the glomerular filtrate. The normal figure quoted for glomerular filtrate is only 15 to 20 mgs. per cent of albumin. I must stress that that figure is in serious doubt, that is to say, there may be more than a 15 to 20 mg. % of albumin in glomerular filtrate. That work is based on glomerular puncture. The levels obtained by Walker, Bott, et al
mg. per cent, I think, but they could pick up concen
per cent

MEMBER The first row, then, is for actual measurements; the last row is a calculated value?

DR. GITLIN· These are calculated values extrapolated to a serum concentration of around 3 1/2 gms. per cent, which is a reasonable albumin concentration.

MEMBER· But these, in fact, are decimal points moved one place to the right.

DR GITLIN· Well, it just happened to work out that way.

MEMBER Why is there such a big difference there? Why is there such a big difference immunochemically? You don't get that average of .35 --

DR GITLIN: You do by electrophoresis. Our figures will average around there. This is pretty well known. I don't want to get into this age-old argument of the tubular reabsorption of protein because that depends on what we would accept as the normal figure for the concentrations of albumin in the glomerular filtrate, and until we know that we can't say very much more. But it would appear that Dr Lauson's figures are correct, and that there appears to be an increase in permeability to albumin irrespective of tubular reabsorption, if we accept those normal figures of 15 mg. per cent.

Similarly with gamma globulin, per 100 ml of capillary filtrate, we find 95.2%

is retained in the peritoneal circulation, 99% in the skin circulation, and 99.5% in the renal glomerulus.

These permeability calculations would indicate one thing very definitely, that even if there were an overall increase in the permeability of the capillary in the skin circulation, peritoneal circulation, or in the glomerular circulation, that there is no real gross permeability; that the increase in permeability is rather small.

MEMBER: Well, then why do you get that tremendous deficit?

DR. GITLIN: Notice that we got a level of around 210 mg. per cent for gamma globulin, just as a passing interest, and the normal would be -- well, we could use the figure of .72% as an arbitrary figure. It probably runs anywhere between 600 and 1,000 mg. per cent -- about 14% of the plasma proteins.

Table 5

	Nephrotic Syndrome	Normal
Albumin Concentration in Serum (mg/100 ml.)	350	3500
Albumin Passing Through Glomeruli per 1 73 sq. meters (mg/min)	6.1*	19**
Ratio: Albumin Passing Through Glomeruli / Albumin Serum Concentration	1.7	0.54
Glomerular Filtration Rate per 1.73 sq. meters (cc/min)	39	127
Ratio Corrected to Normal Glomerular Filtration Rate of 127 cc/min.	5.5	0.54

* minimum value = albumin excretion per minute

** assumed concentration of albumin in glomerular filtrate = 15 mg/100 ml.

Table 5 is directly in line with Dr. Lauson's calculation where, in exactly the same way, we find the ratio of albumin passing through the glomerulus referable to the albumin serum concentration was again what we suspected before, about ten times higher than the normal.

Now, what happens to the albumin when we give it? I think it would explain why, in spite of the fact there is only a slight increase in the overall permeability in the glomerulus, we can get such tremendously low levels of gamma globulin and albumin, particularly the albumin.

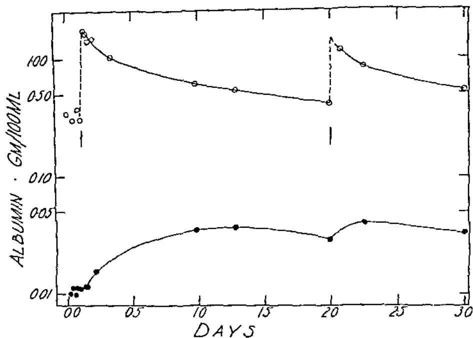


Fig 36 The effect of intravenous administration of albumin on the concentration of albumin in the circulation and in the ascitic fluid

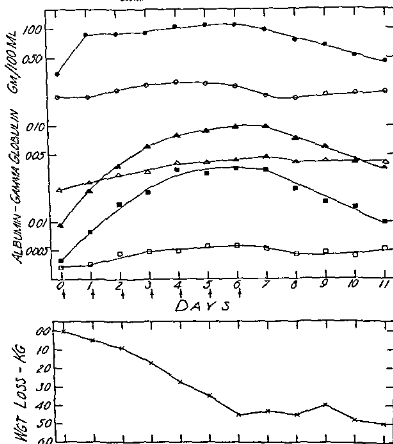


Fig 37 The effect of the intravenous administration of albumin on gamma globulin (open symbols) and albumin (solid symbols) in serum (circles) ascitic fluid (triangles) edema fluid (squares)

Upon giving 25 gms. of albumin to this patient, the serum albumin was elevated to about 1.2 gms. per cent. Notice how rapidly it falls off, in a matter of two days (Fig 36).

Now, our problem was to give a constant amount of albumin over a long period of time so that we could measure the slow changes that take place in the ascitic and edema fluids. We didn't try to give albumin infusions constantly for a period of five to ten days It was a little difficult to do that. What we did was to give 25 gms. of albumin until we reached an equilibrium level so that at a certain period, during every 24 hours, we hoped to reach a plateau where the serum albumin range would be relatively constant. This was the result obtained upon giving albumin for six days, 25 gms. per day, in this particular patient, (Fig. 37). The levels indicated are those found immediately before injections of the serum albumin

A rise in albumin was obtained which then fell off rapidly, which you would expect because of loss in urine, and because of metabolism It took about 6 or 7 days to reach a plateau in ascitic and edema fluid.

CHAIRMAN RAPOPORT: How rapidly does the albumin appear in the urine quantitatively? If you give 25 gms. how many hours or days does it take?

DR GITLIN: It starts to appear immediately. It is impossible to say what is derived from synthesis, or what is derived from what we give, but we can collect an amount of albumin in 24 hours which would be about 90% of what is given in a day.

Now, notice that the level in the body fluids falls off, too. When we consider the dynamic equilibrium that exists between the circulation and the various body fluids, we realize something important Even if you wish to say that there is a one or two per cent increase in capillary permeability in the periphery or in the peritoneal circulation in nephrosis it is completely immaterial, even referable to the edema, because that material is returned to the circulation But when it is lost in the urine, it is irreparably lost to the circulation. So that the slight increase in the overall permeability of the glomerulus to albumin could very easily account for the tremendous drop in the various proteins in the nephrotic syndrome

Now, whether or not there was an increase in the capillary permeability of the skin or in the peritoneal circulation was extremely important to us, because we wanted to know whether edema fluid was representative of the capillary filtrate.

We have just finished this work, and I regret that we have no slides, because there was a delay in making them.

At zero time, we injected Protein-A. In 48 hours, we injected Protein-B, and killed the animal by bleeding him out 15 minutes later, immediately removing a large piece of muscle, the anterior aspect of the thigh. We are able to use this protein to measure the amount of serum left behind in the muscle Measuring this protein, we could then obtain a very simple equation of total protein-A in the muscle, minus the concentration of protein-A that would be contributed by the amount of serum in the muscle We had to use another protein in order to avoid the question of space occupied

by solids, solid materials like collagens, nuclei, and everything else you get in a homogenate. In other words, we had to get the volume of distribution of Protein A and B in our homogenate, and for that we used a third protein, Protein C, and allowed it to equilibrate for 24 hours. We then find that for the total, Protein A is almost twice that of the protein that would be present in the serum in a similar volume of muscle.

MEMBER: What was the purpose of the Protein C?

DR. GITLIN: To eliminate the areas in which Protein A and B could not possibly infiltrate. You have a volume of muscle, you homogenize, and you get a mass, but to know totals, you have to know the volume of distribution of A and B in that homogenate. If someone will grant me that the interstitial fluid is approximately 20% of the net weight I will give these figures:

Table 6

The Concentration of Homologous and Heterologous Plasma Proteins in the Interstitial Fluid of Rabbit Muscle

Rabbit No	Muscle Wet Wt	Serum Concentrations Prot A	Prot B	Volume of Distribution of Protein C	Total found in Muscle Sample Prot A	Prot B	Amount of Serum in Muscle Sample	Amount A in Muscle in Excess of Contribution by Serum	Interstitial Concentration of A	Serum Conc A / Interstitial Conc A
	(gms)	(mg/ml)	(mg/ml)	(ml)	(mg)	(mg)	(ml)	(mg)	(mg/ml)	
845	26 3	6 29	0 608	73 4	10 86	0 276	0 454	8 00	1 52	4 14
846	36 8	7 30	0 696	79 3	15 22	0 178	0 256	13 46	1 84	3 96
847	27 0	0 312	10 39	95 2	0 454	3 90	0 375	0 337	0 0624	5 00
849	21 3	2 74	10 90	90 0	3 15	2 16	0 193	2 62	0 615	4 43
852	32 7	2 38	8 70	80 0	5 76	4 24	0 487	4 60	0 703	3 38
817 a	18 4	3 44	2 54	93 6	3 18	0 467	0 184	2 55	0 693	4 95
817 b	17 9	*	*	65 0	3 25	0 660	0 260	2 36	0 659	5 21
817 c	22 0	*	*	93 6	3 09	0 373	0 147	2 58	0 586	5 88

The concentration of plasma protein present extravascularly, on the basis of 20% volume of distribution would be about one-third the concentration in plasma. If you want to take 10%, well, it would be even higher, I guess. It would be 1: 1.5 to 3. In other words, there is a large amount of plasma protein outside the circulation, in the immediate area of the capillary

This does not invalidate Starling's hypothesis one iota, but this does corroborate the analyses which were obtained by Dr. Drinker -- he always assumed that the protein present in the lymph was representative of the interstitial fluid. This was checked by Dr. Weech and he came up with the same idea, on the basis of analysis of edema fluids referable to lymph fluids It would appear that this does obtain· that the interstitial plasma protein concentration is equivalent to the lymph protein concentration.

We are running into a sort of paradox here If the protein concentration ratio in the normal is 1.3, in the nephrotic, the concentration is about 1·30. Dr. Weech postulated on the basis of this possibility that the capillaries were less permeable to the plasma proteins in the edema states This is not necessarily so. This you can easily see On the basis of Starling's hypothesis, you have water, plus a certain amount of protein, coming out at the arteriolar end of the capillary and water is reabsorbed at the venous capillary But the plasma protein gradient is outward all along the capillary. So that there is a certain amount of lymphatic drainage, and that drainage is normally not very high The equilibrium value of 1·3 is representative of water being reabsorbed and plasma protein not being reabsorbed.

Now, let's consider the disequilibrium state of the capillary. There are .3 gms. of albumin in the serum; water comes out all along the capillary until a point is reached where the tissue pressures begin to rise. The tissue pressure rises, and much of the fluid is taken off by the lymph. With a rise in tissue pressure, some of the water does go back But this rise and flow would explain what would appear to be the paradox between the nephrotic and the normal patient In other words, the edema fluid is much more representative of the capillary filtrate than would be the normal interstitial protein levels

I think I am going to give up at this particular time because I think it is immaterial except from the purely academic point of view at this stage of our knowledge as to what the actual capillary permeability is in the skin or the peritoneal circulation other than to show that there is no gross increase in capillary permeability.

The other point that I wish to make is that the data of others on protein concentration in lymph is consistent with our direct measurements of interstitial fluid protein concentration; this lymph fluid is apparently representative of the interstitial fluid. In edema states with such marked depression of the oncotic pressure, the edema fluid represents much more closely capillary filtration.

MEMBER· We differ in definition of capillary permeability

DR GITLIN: Before we say another word, let us say what we mean by capillary permeability. What would you say capillary permeability represents?

MEMBER: I would measure capillary permeability only with water.

DR. GITLIN. All right, I will accept that. What would be your equation for capillary permeability? Would it also depend upon the concentration gradient?

MEMBER: No.

Dr. GITLIN: Then that is where we differ. The definition of permeability of a membrane depends upon the definition of cross section area and concentration. In the patient with low oncotic pressure, assuming simple diffusion without bulk flow, you have a higher concentration of water relative to protein than you would normally. If we have an albumin concentration of X on one side and water on the other side of the membrane, water moves with the diffusion rate of D-1. Decreasing the albumin concentration reduces the rate of diffusion of water across the semipermeable membrane.

Now, does that mean the first membrane is more permeable? No. It means only that it is based on the gradient of water between these two concentrations.

DR KAPLAN: There is no argument about the question that osmotic pressure has an influence, but the idea that capillary permeability is measured only by the passage of protein is an untenable one For you can have water passing through a membrane which is more permeable to it at many times the speed without protein leaking through it. Normal skin capillaries will retain a molecule of about 1000 molecular weight, not considering all the charged effects, and albumin has a molecular weight of about 60,000, that means between 1,000 and 60,000 you can have an increase of capillary permeability to water Actually, in membrane experiments, 28 times as much water will go through before protein starts to leak. And this explains why you apparently, in your figures, show that you have less protein in the outside fluid, for you have more water to dilute it into.

DR. GITLIN: That may be so. I will not argue on the possibility of the capillary membrane being permeable to water or permeable to protein, when you have an increase in permeability it is still possible to increase the diffusion to such an extent that the water will go through at a faster rate without increasing protein. With that I have no argument. But we were talking about the permeability of protein. I am not willing to go into the permeability of water in the nephrotic syndrome We must remember that water has a certain concentration; I think it was Crawford one time, at Old Point Comfort, who said he preferred to use serum water concentration, and everybody jumped on him I don't think it is a bad idea at all. You don't have to postulate increase in permeability of the capillary to the water alone. So I won't argue. Your hypothesis is entirely feasible, but there is no need to hypothesize it.

DR. COOKE: Dave, this bothers me a lot, this thing we are dealing with, this *water concentration*. The normal concentration of water is about 940 gms. per liter of plasma, and we have some figure which you will have to make up because I don't know it. It will probably be closer to 980, would you say?

DR. GITLIN: Yes, that is close.

DR. COOKE: Now, we have seen patients with a water concentration of somewhere in the vicinity of 680 gms. of water per liter of serum. As I understand your way of thinking here, we should have an awful lot more water pouring back into this fellow's plasma.

DR. GITLIN: Not necessarily, if this is an equilibrium state. We would not be talking about a disequilibrium state. By the way, you are taking water concentration in terms of total solids, and then subtracting. In permeability, it should be referred to solutes of differing permeabilities.

CHAIRMAN RAPOPORT: Dr. Pressman will continue the immunochemical discussion by presenting some material on immunologic mechanisms, with particular reference to the role of nephrotoxic sera.

DR. PRESSMAN: I think I had better play it safe and talk about renal disease when I talk about nephrotoxic serum, and it is known that renal disease can be produced by anti-sera prepared against kidney

We have gone on to show that the antibodies in the anti-kidney serum will actually localize in the kidney, and we have done that by radio-iodinating anti-kidney serum . . . that is, treating the anti-serum with iodine, containing radioactive iodine. The iodine combines chemically with the protein and acts as a label which follows the distribution of the antibody. Radio-iodinated proteins have been mentioned here several times already, and I have been asked by members of the audience about radio-iodinated proteins, so I thought it might be well to comment on the radio-iodination processes.

The process of radio-iodination is a simple one. Radio active iodine, which is obtained from Oak Ridge in a carrier-free state is mixed with carrier iodide ion. This is treated in acid solution with nitrous acid to give free iodine, i.e., molecular iodine, and then this acidic iodine solution is neutralized by adding base and buffer. Then the mixture is added to the protein, the tyrosine rests in the protein combine with the iodine, producing an iodine-carbon bond. When an anti-serum prepared in rabbits against rat kidney is iodinated and then injected into a rat, there is a localization of radioactivity in the kidney, apparently due to anti-kidney antibodies. This is shown by the fact that there is more radioactivity localized in the kidney of an animal receiving radio-iodinated anti-kidney serum, than in an animal receiving radio-iodinated normal serum proteins. There is also a greater accumulation of radioactivity in the liver, and some in the lungs of these animals receiving an anti-kidney serum, due to some cross raction of anti-kidney antibodies with these organs.

There is a very slight pick-up of iodine in the thyroid, from these radio-iodinated proteins. The thyroid picks up iodine which has been separated from the protein through metabolic processes. This iodine does not disassociate from the iodinated protein but becomes available as the whole protein molecule is metabolized.

The localization of the anti-kidney antibody takes place in the glomerulus of the kidney Radioautographs of sections of kidneys of mice receiving the radio labelled anti-kidney serum or radio labelled anti-albumin control serum, both containing the same amount of radioactivity, are shown in Figures 38 A and B. The section in 38A is of the kidney of a mouse receiving anti-kidney serum. There is a punctate distribution of the radioactivity through the kidney section.

These spots correspond to the glomeruli in the original section. This is a poor section, and was taken through the cortex primarily, so that we have glomeruli distributed throughout.

In Fig. 38B we have the control section of the kidney. There does not seem to be any high accumulation of radioactivity in the glomeruli of the control kidney.

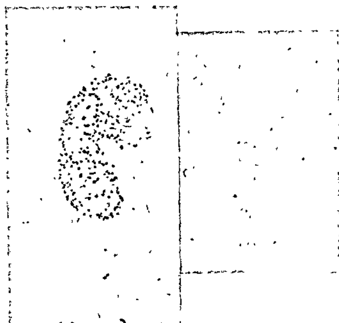


Fig 38. Radioautographs of mouse kidneys following injection of labelled (A) anti-kidney serum, (B) anti-albumin control serum.

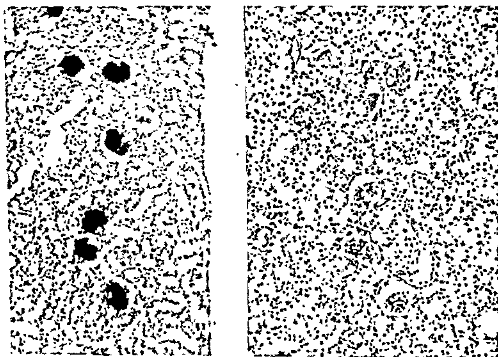


Fig 39: Radioautographs of kidney sections from mice following injection of labelled (A) anti-kidney serum, (B) control serum.

Figures 39 A and B show another pair of sections. The proteins here were labelled with sulfur but that doesn't make any difference. These slides were prepared by placing the kidney tissue on the radioautographic plate; the section is permitted to remain on the plate several days, then the plate is developed, and then subsequently the sections were stained to show the structure of the kidney section Figure 39A shows the section from the animal receiving the anti-kidney serum, and Figure 39B shows the section of an animal receiving a control serum. You can see there is a very strong localization of radioactivity in the glomerular tuft. Bowman's capsule is free, and the tubules seem to be free of radioactivity Notice there is no corresponding localization in the glomeruli of the control animal.

The localization of the antibody in the glomeruli fits in well with the observation of others, for example: Heymann et al., (Science 73: 385, 1950) have shown that the cortex contains much more of the antigen structure for producing the nephrotoxic anti-serum, and Solomon and co-workers (J. Exper. Med., 90. 267, 1949) have shown that isolated glomeruli will absorb out the nephrotoxic factor of anti-kidney serum, and Krakower and Greenspan have shown that anti-serum prepared against other kidney structures are not nephrotoxic.

In our experiments, we have found that the localization of the anti-kidney antibody takes place very rapidly The blood is essentially cleared of antibody as it flows through the kidney Kay (J Exper. Med , 72; 559, 1940) carried out a similar experiment in vitro in which he perfused a rabbit kidney with nephrotoxic serum and showed that the perfusate which came out of the kidney no longer was nephrotoxic.

Now, the way nephrotoxic sera are often prepared, by injecting a rabbit with the whole kidney homogenate, produces a multiplicity of different antibodies. The kidney homogenate, which is injected, is composed of a good many different proteins. There are the proteins which are associated with glomeruli, those with the tubular surfaces, those with the contents of the various cells, and antibodies are produced against all these different antigenic substances However, not all of these antibodies are nephrotoxic antibodies. If one radio-iodinates some anti-kidney serum, and adds it to the soluble part of a kidney homogenate (this is a common way of determining the "titer" of an antiserum) one obtains a precipitate of the anti-kidney antibody, and the soluble materials. However, the antibodies which are precipitating are not those antibodies which localize in the kidney. This can be shown by injecting the supernate from the mixture of radio-iodinated anti-kidney serum, and the soluble part of the homogenate into a rat The localizing activity is just as great as it was before absorption with the soluble part of the kidney homogenate.

However, if one treats the radio-iodinated antiserum with the insoluble parts of the kidney homogenate one absorbs out, quite effectively, all of the localizing antibodies So here we have at least two types of antibodies, those prepared against the soluble part, and those prepared against the insoluble part, and it is only the antibodies against the insoluble part that localize

Now, I think this observation is rather important in connection with measurements of titer of anti-kidney antiserum. It is obvious that if one measures the titer of an antiserum by the precipitin test, one is only measuring the titer of the antiserum with

respect to antigens which are not important in the localizing action of the anti-kidney serum. Moreover, rabbits give a very widely varied response to antigens. Different rabbits will efficiently produce antibodies against one component in a mixture of antigens, whereas another rabbit may produce more antibodies against a different component. So it is fairly obvious that there need be no correlation between the production of nephrotoxic antibodies or localizing antibodies and the production of antibodies against the soluble material.

In the experiments which we carry out, the study is made in vivo, that is, we inject the anti-kidney antibody and we determine where it localizes. Obviously the antibody must come in contact with the antigen in order to localize. Other antibodies that are injected are the non-localizing antibodies. They are prepared against components of the kidney tissue, which cannot be reached by antibodies with circulation, for example, against cell contents They will react with components of the kidney tissue, in the in vitro experiment where the cells are broken but they just can't get through to the antigen in the intact cell.

Some antibody molecules may be picked up non-specifically by these cells, and thus get into the cells. They may then react with antigen - but these will be relatively few as compared with the antibodies which are localized on structures which are in good contact with the blood stream. We think the localizations that we observed do take place on the vascular bed, i.e. those structures which are in intimate contact with the blood stream. This would also explain the very rapid localization which we have observed.

Recently, two English investigators, Hill and Cruikshank, (Brit. J. of Exp. Path. 34: 27, 1953) have published reports on studies with anti-kidney serum that are somewhat different in approach. Their method of study was the use of the fluorescent-labeled antibodies which have been developed by Coons of Harvard. In those experiments, anti-sera were prepared against the whole kidney, or parts of the kidney, coupled with fluorescene and used as a histologic stain for staining kidney sections. The antibody sits down where antigen is located in the section and fluorescence can be observed there under the ultraviolet microscope.

Now, Hill and Cruikshank showed also that there were several components in anti-kidney serum. One was directed against the tubular cell contents and stained them. These are probably the antibodies which did not localize in vivo in our experiments. In in vitro experiments, the tubule has been sectioned, and the contents are now open for contact with the antibody

They also report the presence of another antigen, which seems to be present in the basement membrane of the glomerular tuft and in the basement membrane of the tubules. We did not observe any localization of radioactive antibody in the tubular area in vivo; there may have been some, but it was at a much lower level than we were able to pick up

We have been able to carry out purification procedures in order to isolate antibodies which would localize more efficiently in the kidney.

In this procedure, we add the insoluble part of the kidney homogenate to the radioactive antiserum, centrifuge down the sediment which now has a radioactive antibody associated with it. Then by heating to about 60° we can dissociate the radioantibody from the sediment to give us a specifically purified preparation in the supernate. When we used kidney sediment for purification, the eluate localizes to a much higher degree in the kidney than did the unpurified material previously.

Antisera prepared against other tissues have been reported to be nephrotoxic. For example, Chicamutso (Folia Endocrin. Japonica 16: 85, 1940) prepared anti-rabbit lung serum in ducks and demonstrated nephrotoxic activity in that antiserum. Beatrice Seegal (J Exper. Med. 84: 211, 1940) has been studying antiserum prepared against placenta. She found placenta activity, that is, the antiserum would produce abortion, but these anti-placenta sera were very active in producing renal lesions, also.

Todo studied anti-liver antibodies and observed nephrotoxic activity. Strehler (Schweiz. Med. Wochenschrift, 81: 109, 1951), studied rat aorta and found nephrotoxic activities. Some of these investigators also reported toxicity against the organ against which the antibodies were prepared.

We have studied similar sera, anti-aorta, anti-lung, anti-liver sera, which we prepared and also an anti-placenta sera prepared by Dr. Seegal. In all these sera we have been able to show localization of radioactivity of the radio antibodies in the kidney.

DR. SLATER: Dr. Pressman, this tissue sediment that you are combining with the radioactive antiserums, is that kidney tissue sediment, or some other organ that you are using?

DR PRESSMAN We can use any sediment we wish to If we have an anti-kidney serum and we use the sediment from kidney tissue and we carry it through this process and we get good kidney localizing activity.

MEMBER: Is this just when you ground it up as a homogenate?

DR PRESSMAN: We homogenize the kidney tissue, centrifuge down the insoluble material, and wash the insoluble material several times in saline

MEMBER: At what speed do you centrifuge?

DR PRESSMAN: This is brought down at low speed

MEMBER: This is the whole material?

DR. PRESSMAN: Yes -- the sediment -- a very crude material The whole kidney is homogenized, and the material that comes down at slow speed is classed as the sediment If we use lung anti-serum and absorb with kidney tissue, we bring down kidney localizing activity and very little lung localizing activity. Now, if we take the same antiserum and absorb it, with the lung sediment, we will bring down lung and kidney localizing activity

We have carried out a finer fractionation, by pushing tissue through a tissue press. Then we have a residue which stays behind on the screen, and material which comes through. The residue which is on the screen is composed of the large blood vessels and connective tissue, and the material that comes through, we call the cellular fraction. We carry out two preparations, one with the kidney cellular material -- let's not call them cells or blood vessels, let's call them cellular material -- and blood vessel preparations. The same serum is purified with these two preparations, and the blood vessels seem to purify the liver localizing activity and the kidney localizing activity much better than the cellular preparation

Now, if we purify an anti-kidney serum with liver preparations, we find that the blood vessels from liver will purify the kidney localizing activity to a much greater extent than the cellular fraction -- and in the various tissues that we have studied it has always been the blood vessels of the tissue which will purify the kidney localizing activity.

In the case of anti-lung serum, the whole serum shows localization in the liver and in the kidney and in the lung. If we carry out a purification process with lung blood vessels, we find that we purify out liver and kidney localizing activity but not much lung localizing activity. If we purify by the use of the lung cells, we find we do not purify as much of the liver and kidney localizing activities, but we purify out more of the specific lung localizing activity. In all these localizations of these antibodies, we feel strongly that the localization is taking place on the vascular bed - and in the case of the lung we would say that there is a component of the lung tissue, the lung cell, which is close to the blood stream and which composes part of the vascular bed, so that there is good contact with blood and good localization of the radio-antibody rapidly, as we have observed.

In the case of anti-liver serum, we obtained the best purification of the liver localizing material and the kidney localizing material with the liver blood vessel preparation.

From these experiments, I think we can say that anti-kidney serum antibodies which localize in the kidney from these other sera are antibodies which are prepared against the blood vessels of these tissues. There are component antigens in common in the blood vessels of these tissues, and in the kidney tissue. However, the kidney may well have components which are unique for kidney

Now, actually there appears to be a fairly great heterogeneity of these antibodies which do localize in the kidney, and some evidence for this is the neutralization of antibodies before and after purification. In this neutralization process, we have prepared soluble materials from kidney tissue, after the methods of Cole and his co-workers (Proc Soc. 77; 498, 1951). Cole digested kidney tissue with trypsin, obtained soluble materials which would remove the nephrotoxic activity of anti-kidney serum. We have studied similar preparations and have found that these preparations will neutralize the localizing antibodies. The soluble material added to an anti-kidney serum will greatly reduce the localizing activity of the anti-kidney serum. However, there is quite a difference in the neutralizing activity of this soluble kidney antigen in neutralizing localizing activity of the unpurified anti-kidney antibodies and the

purified kidney antibodies. The unpurified antibodies are neutralized much more efficiently.

This purification process that we run through is, of course, a very crude one. We pick up a fair amount of radio-antibody on the sediment -- and then we elute. We just elute a relatively small fraction of the radio-antibody which was picked up. It is probably the antibodies of lowest combining power that are eluted. We have found that the antibodies in whole serum are fairly efficiently neutralized by the soluble material; more so than are the antibodies which we have eluted in the purification process. Although the purified antibodies combine strongly enough to give localization, they do not combine strongly enough to be neutralized by this soluble material which is obtained by the digestion of kidney tissue.

MEMBER: Just what do you mean by the term "purifying kidney localizing activity"?

DR PRESSMAN Well, we purify kidney localizing activity -- we start with an anti-kidney serum which localizes to the extent of 1/2% of total radio-activity injected per gram of kidney tissue The low value is due to the large amount of non-specific proteins present in this anti-serum, and these all bear the same radioactive label. When we carry out this absorption and elution process, we end up with a material which will localize to the extent of perhaps 10% of the radioactivity injected per gram of kidney tissue

CHAIRMAN RAPOPORT: In all of your statements on localizing activity, are you using it synonymously with the ability to produce damage?

DR PRESSMAN I am just talking about localization, I can't talk about damage. All our experiments are carried out at low levels of antibodies which would not be expected to produce nephrotoxic effects Known nephrotoxic sera do not show kidney localization Dr Seegel had administered our serums in larger amounts and found them to be nephrotoxic Dr Heymann's duck serum shows localization. We have carried out experiments with nephrotoxic anti-serum given to us by Dr. Seegel -- these are anti-placenta serum -- and they show the localization. The properties of our localizing antibodies which we have been studying are quite parallel with the properties of the nephrotoxic antiserum, especially as listed by Smadel (J. Exper Med., 64: 921, 1936) in his experiments The reason for studying anti-kidney serum in the first place was that we were interested in localizing radioactivity in specific tissues. Here was an antiserum - which was physiologically active and in order for it to be physiologically active, we felt that it had to go to the tissue in order to affect it. I am sure that the nephrotoxic antibodies are in our localizing antibodies, - however, there may be localizing antibodies which we are studying which are not nephrotic.

DR HEYMANN. May I ask one thing - I remember the beautiful pictures of the English authors, Hill and Cruickshank - those beautiful fluorescent zones on the tubular basement membrane; what is the reason that your radioactive iodine label didn't show that?

DR PRESSMAN: The reason that our radioactive material does not show up in

the tubules is that it just didn't get to the basement membrane in the tubules in high enough concentration.

MEMBER: Have you ever tried applying antibody to sections in order to see what they did?

DR. PRESSMAN: Well, we have thought about it, but we haven't carried it out because we thought we would get the same results that Hill and Cruickshank have since reported, and we were interested in the in vivo localization. We knew that there were a lot of antigens that we were missing, and they were sitting there in the kidney, and we weren't especially interested in those not important in localization.

MEMBER: Didn't they inject the antiserum intravenously?

DR PRESSMAN: No. They just take a section of a kidney, and then use the fluorescent antiserum as a specific histological stain.

MEMBER: For a digest of the kidney, do they use soluble antigens?

DR. PRESSMAN. Yes, it neutralizes the localizing activity.

MEMBER: Does this mean that the work of Greenspon has been discredited, that the basement membrane was found to contain the antigen?

DR PRESSMAN: No, not at all. I am not sure that it necessarily has to be the basement membrane from Greenspon and Krakower's work. Greenspon and Krakower have carried out their studies by determining the antigenicity of the various renal materials that they isolate.

MEMBER: Was it with rats or rabbits?

DR. PRESSMAN: They worked with dogs and inject the material into rabbits. Their work is clouded by the individual variation of the rabbits with respect to the antibody response, and also, by the biologic variation in the actual assay. I think that it is more straightforward to produce an antiserum, determine its constants in terms of localization, and then absorb them out with various preparations. The antigen responsible for localization may well be in the basement membrane. We talk about the vascular bed as those structures which are in contact with the blood. Those structures in good contact with the blood stream remove the localizing antibodies rapidly. And in the various tissues and organs in which we do get localization, we feel it is due to some component of the cells, special cells, which are in contact with the blood stream at this vascular bed area. Different organs remove different substances from the blood and produce substances which they liberate into the blood. These special cells may well have specific surfaces. In the case of the kidney, the fenestrata described by Hall, may well be the antigen which is important here. It seems remarkable that the kidney should be able to have so much blood flowing through it and be able to clear this antibody as rapidly as it does. The kidney does all kinds of things rapidly, and here it may be this fenestrata with all its surface which is being very effective in removing the kidney localizing activity.

MEMBER: Did I understand you haven't studied localization of the antibody given in doses that are large enough to produce the disease?

DR. PRESSMAN: Well, we have injected doses large enough to produce the disease, as shown by others, but we did not look for disease. What we tried to do is actually saturate the kidney with antibody. When we injected large amounts of anti-kidney serum (15 mg/rat) we obtained a decrease in the amount of localization of the radioactive material, but we also observed a very great decrease in the blood level of radioactivity. These observations would indicate cytotoxic effects.

CHAIRMAN RAPOPORT: Are you implying that gamma globulin can act as a blocking agent?

DR PRESSMAN The antibody is in the gamma globulin fraction

CHAIRMAN RAPOPORT: Did you mix normal gamma globulin with your anti-body gamma globulin?

DR PRESSMAN: Yes, in order to keep total globulin administered constant.

MEMBER: Has anybody worked with animals, with what you would call your purified antibody material that has been absorbed with sediment and consequently will only react with intracellular kidney material?

DR PRESSMAN No

DR. METCOFF: What happens if you prepare an antibody just against glomeruli?

DR PRESSMAN I know Cruickshank prepared antibodies just against glomeruli, and of course Krakower and Greenspon prepared antibody against just glomeruli. These are nephrotoxic sera, so that glomerulus definitely has the antigen which is responsible for the development of the nephrotoxic antibodies.

CHAIRMAN RAPOPORT: Dr. Baxter, do you want to continue the discussion?

DR BAXTER: Dr Howard Goodman and I have recently started some work on experimental nephrotoxic serum nephritis. We have been interested in determining where the antigen is which is responsible for the production of the nephrotoxic anti-body and the possibility of getting the antigen into soluble form so that it might be purified and characterized. We have also been interested in the clinical picture and course of the disease, and in the mechanisms of the lipemia, hypoproteinemia, and proteinuria which occur

In preparing our nephrotoxic antiserum, we have divided rat kidney tissue into glomerular and tubular fractions, employing a method similar to that of Solomon et al. (J Exper Med, 90 267, 1949), and rabbits have been immunized by repeated intra-peritoneal injections of these fractions The rabbits receiving glomerular material developed potent nephrotoxic sera, as was expected from the observations of other

investigators. A milliliter and a half of these sera have consistently produced moderate to severe proteinuria in 150 gm female rats, the proteinuria beginning immediately following the injections. Not only did the rabbits receiving glomerular material develop nephrotoxic antibodies, but the rabbits receiving the tubular material also were observed to develop sera which produced proteinuriaund renal injury in rats similar to that produced by sera from the rabbits receiving glomeruli. While the tubular material may have been contaminated with a small amount of glomerular material (soluble or insoluble), we are inclined to regard these results as indicative of the presence of nephrotoxic antigen in nonglomerular tissue as well as in glomerular tissue. This concept is supported by the results of protection experiments in which it has been observed that tubular and even medullary tissue is able to absorb the nephrotoxic antibodies from nephrotoxic serum Liver and striated muscle in equal amounts did not inactivate the serum. Furthermore, protection experiments have indicated that not all of the antigen remains in the insoluble residue of kidney homogenates. Some is present in saline extracts after centrifugation in a Spinco at 27,000 RPM for 1 hour.

Many of the rats receiving the nephrotoxic serum developed hypoproteinemia, edema, and marked elevations of plasma lipids, somethimes reaching levels of 10,000 mg per cent but more commonly 1000 to 3000 mg per cent Chemical analyses by Dr. Bragdon indicated that the neutral fat was greatly elevated, while cholesterol and phospholipid were moderately elevated. We have observed that injection of 0.2 mg. of heparin into these rats with elevated plasma lipids causes clearing of the visible lipemia and a fall in total lipid levels almost to normal. The vascular system of some of the rats with hyperlipemia was perfused with saline and sections of the hearts stained for fat. Minimal lipid deposits were observed in the valve walls in a few cases. I was interested in hearing Dr. Heymann's discussion of the lipoprotein pattern in his animals. Dr. Boyle has been good enough to study a few of our sera by ultracentrifugation and has observed a moderate amount of abnormal S_f 20-100 material.

In starting work on this problem we were very much interested in the experiments of Cole, Cromartie, and Watson (Proc. Soc. Exper. Biol. & Med. 77: 498, 1951) in which they were able by trypsin digestion of kidney homogenate to get into the supernatant fluid (after ultracentrifugation) something which would inactivate nephrotoxic serum so that it was no longer capable of producing renal injury In preliminary experiments, they thought that this soluble substance was non-antigenic, and they presumed that it was something like a haptene which would combine with antibody but not act as an antigen. As you know, the antigen is supposed to be in the glomerulus - probably in the glomerular basement membrane, and in homogenates of kidney, it has generally been thought to remain mostly in the insoluble residue. So we were interested to see if we could repeat the observations of Cole et al In many experiments we have homogenized 25 to 50 gm of kidney with an equal volume of saline, added 150 to 300 mg. of trypsin, adjusted to pH 8-8 5 and incubated at 37° for 3 hours. Then the digest was heated to 56° for 30 min. and spun in a Spinco at 27,000-30,000 RPM for 30 min. or longer. The supernatant fluid was drawn off and used in protection experiments. In control experiments, we have used similar digests of liver and skeletal muscle. We have found that 2 ml. of the supernatant fluid from the kidney digests are regularly able to remove the nephrotoxicity of 1 5 ml. of nephrotoxic serum when the two materials are incubated together and then injected. Furthermore, intravenous injection of the supernatant material as long as 10 minutes prior to injection of the nephrotoxic

serum prevents renal damage by the serum. The supernatant material obtained from digestion of liver and muscle have not prevented the nephrotoxic effect of the serum. Thus we have confirmed the observation of Cole et al.

We have made some efforts to characterize the protective factor in the supernatant fluid from kidney digests. The material appears to be nondialyzable, stable to 60° C but destroyed by 100° C, and is destroyed by acidification below about pH 4. Thus far the material has resisted destruction by trypsin and chymotrypsin. We are planning to go ahead with attempts to purify the material by fractionation, and to test the effects of various specific enzymes on it We are also interested in separating anatomical portions of kidneys -- glomeruli, tubular material, and medullary material -- in order to see if the protective factor can be obtained by digestion of these fractions

Experiments are now in progress to determine whether the protective supernatant material from kidney [illegible] of stimulating the production of nephrotoxic serum whe[illegible] rabbits). It is too early to draw definite conclusions but the results of an early bleeding of the rabbits failed to show that the supernatant fluid from the digests was antigenic, while various portions of kidney tissue and even saline extracts (after ultracentrifugation) did appear capable of producing nephrotoxic sera Further work is required on the quantitative aspects of these experiments

III Experimental Nephrotic Syndrome

CHAIRMAN RAPOPORT: Dr. Heymann will speak first on The Experimental Nephrotic Syndrome in Animals.

DR. HEYMANN: First I would like to discuss experiments that refer to the pathogenesis of nephrotic hyperlipemia. One group of experiments was concerned with the importance that the liver may play in the development of the nephrotic hyperlipemia Rats were subjected to subtotal hepatectomy, which removed sixty to eighty per cent of liver tissue Immediately thereafter they were injected with nephrotoxic serum (N.T.S.) Blood chemistries were obtained twenty-four and forty-eight hours thereafter. It was found that the injection of blank rabbit sera obtained from healthy untreated rabbits in amounts corresponding to those used when nephrotoxic sera was injected, does not affect serum protein, total lipid, or cholesterol values in hepatectomized rats. It was furthermore found that the injection of nephrotoxic sera in hepatectomized rats was followed by a slight but definite hyperlipemia. The hyperlipemia noted in control rats that were injected with the same nephrotoxic serum but that were not hepatectomized was more statistically significant. Control experiments were carried out in which the spleen was removed instead of the liver No difference was noted in the degree of hyperlipemia that developed in splenectomized and not splenectomized animals injected with the same N.T.S. I believe that we may conclude from these experiments that the liver is involved in the mechanism that elicits the nephrotic hyperlipemia. We then investigated the importance of the kidneys for the development of the nephrotic hyperlipemia. In these studies bilateral nephrectomies were performed instead of hepatectomies. Immediately thereafter N.T.S. was injected and blood lipid values were obtained after forty-eight hours. Bilateral nephrectomy alone gives rise to a slight hyperlipemia. This was described some years ago.(1) No additional rise in blood lipid values was observed when N.T S. was injected in bilaterally nephrectomized rats, whereas the same N T S. produced the usual marked hyperlipemia in not nephrectomized rats. In other words, without kidneys no hyperlipemia. We then tried to produce unilateral nephrotic disease in a group of seventy-two rats; this was done by clamping the renal pedicle on the left side for twenty-five to thirty minutes after the injection of N.T.S From seventy-two rats so treated, twenty-eight developed unilateral disease. This was checked in each animal by histological examination of the kidneys. Rats with unilateral nephrotic renal disease do develop some hyperlipemia which is, however, definitely less marked than that observed in rats with bilateral nephrotic disease This points toward a certain quantitative relationship between the amount of nephrotic renal tissue and the degree of the hyperlipemia. It also teaches us that the presence of healthy renal tissue does not prevent the development of the nephrotic hyperlipemia. A relationship between nephrotic renal mass and degree of hyperlipemia was also noted in another group of studies in which unilateral nephrectomy was performed in rats with unilateral and bilateral nephrotic renal disease. Within three to four days after the removal of the right sick kidney blood lipid values returned to normal. If unilateral nephrotic renal disease had been present when right nephrectomy was performed in rats with bilateral nephrotic disease, the blood lipid values decreased somewhat but never came down to normal range. In other words, again, a

(1) Heymann, W and Clark, E.C. Pathogenesis of Nephrotic Hyperlipemia. Am. J. Dis. Child. 70: 74, 1945.

certain correlation was found between the amount of nephrotic kidney tissue and the degree of hyperlipemia.

We have been also interested in studying the lipoprotein pattern of nephrotic rats. Too little blood is available to carry out such studies with chemical methods. Our studies were carried out by Dr. Lena Lewis from the Cleveland Clinic Research Division, using an ultracentrifuge method. The method that Dr. Lewis uses(2) differs only slightly from the original Gofman procedure. It was applied to eleven nephrotic and five control animals. According to these studies the increase is found chiefly in the beta-2 and beta-1 lipoproteins. Some increase in alpha-2 lipoproteins was also noted while alpha-1 lipoproteins were correspondingly decreased (Table 7).

Table 7

Ultracentrifuge Lipoprotein Pattern, Rats, % Distribution, Density 1.21.

-S	70	40-70	30	23	10-20	4
5 controls	29		10	4	12	45
11 nephrotic rats	42	11	14	14		19

These results correspond quite well with what has been found in nephrotic children. The -S 40-70 fraction is the one that according to Gofman is of importance for the development of arteriosclerotic lesions and in nephrotic hyperlipemia this fraction is increased. This might be worth while noting, remembering the instances of atheromatous lesions observed in nephrotic patients.

Another group of studies has been concluded which I would like to report on briefly. These were concerned with the behavior of serum complement in the nephrotoxic renal disease of the rat injected with nephrotoxic rabbit sera They were carried out by Dr. A Stavitzky from the Department of Microbiology. An immediate drop of complement titer has been observed regularly in all animals that did develop renal disease. It was observed thirty minutes after the injection of N T.S After forty-eight hours the values returned to normal and remained normal all through the course of the continuing disease When serum of rabbits that had been treated with rat kidney extract was used, and when this serum did not produce renal disease, a drop in complement was, however, occasionally observed. This reaction, we believe, is most likely due to the presence of other than renal antibodies. This assumption has been substantiated by finding that in bilaterally nephrectomized rats the injection of N T.S may also be followed by a complement drop. The presence of some extra-renal antibodies has also been suggested by a series of absorption experiments When N.T.S. were treated with rat kidney extracts these sera failed to produce renal disease, but could be followed by a short

(2) Lewis, L.A , Green, A. A. and Page, I. H. Ultracentrifuge Lipoprotein Pattern of Serum of Normal Hypertension and Hypothyroid Animals. Am. J. of Physiol. 171· 391, 1952.

term complement drop. When N.T.S. were treated with rat plasma they produced both complement drop and nephrotoxic renal disease. We believe then to have shown that other than renal antibodies are present in N.T.S., which may lead to a temporary complement depletion but that renal antibodies are probably always involved in this mechanism when nephrotic renal disease ensues in that it never has been missed in such instances.

When N.T.S obtained from rabbits is used no latent period is observed. When N.T.S. obtained from ducks is used it is common to note a latent period. We, therefore, have studied in another group of experiments the localization of iodinated duck anti-kidney sera in the kidneys of rats three days after the duck anti-kidney serum had been injected intravenously. This was done with a duck serum that had a latent period of from five to seven days. The results were compared with the localization of an anti-kidney serum obtained from a rabbit that as usual had no latent period. The localization was expressed in per cent of the injected radio activity which localized in one gram of tissue three days after the injection. This work was done by Dr. David Pressman with the methods that he has described(3). It was found that both anti-kidney sera prepared either in the duck or in the rabbit localized to a greater degree in kidney (and in liver) than the normal control sera. The different biological activities of the duck and rabbit sera, therefore, cannot be attributed to any inability of duck anti-kidney serum to localize in the rat kidney. This is well in accord with the old theory of Kay (4). The only addition that Dr. Pressman proposes to Kay's old theory is that complement fixation is needed for the ensuing cellular injury and that complement fixation occurs immediately when rabbit serum is used, whereas when avian sera are used, it only occurs after antibodies to the injected duck sera have been produced. This addition is in accord with our complement studies in the rabbit - rat disease previously mentioned, and with preliminary results thus far obtained after the injection of N T S obtained from ducks.

To close, I would like to report on studies that were concerned with the production of the nephrotic syndrome in dogs. Thus far we have used twelve puppies. These were injected with sera obtained from rabbits that were treated with dog kidney extracts. They were treated according to the same schedules and procedures that have been described previously for the rat disease. To start with the dogs were injected also with the same dose that rats were injected with, namely, with 1.4 cc. of serum per gram of kidney. This dose was divided in three daily doses given on three consecutive days. If no proteinuria ensued this procedure was repeated once or even twice. Total amounts of 50 to 60 or more cc. of sera were thus used. Serum toxicity that often led to death of the dogs immediately after the injection was more frequently encountered in dogs than in rats and was successfully avoided by injecting the puppies one hour prior to the injection of serum with 0.5 to 1.0 cc. of pyribenzamine intramuscularly (5) Blood chemistry values for normal healthy puppies were first obtained and were found to be different from the control values established in rats. In healthy puppies

(3) Pressman, D. and Eisan, H. N. J. Immunol. 64: 273, 1950 and 67: 15, 1951.
(4) Kay, C. F. The Mechanism of Glomerulonephritis; Nephrotoxic Nephritis in Rats. Am. J. Med Science. 204· 483, 1942.
(5) Winternitz, W W. and D. B. Hackel. Effect of Antihistaminics on Experimental Nephritis in Rabbits. Proc. Soc Exper Biol. Med., 78: 294, 1951.



Fig. 40 Glomerulus of normal dog. Hematoxylin and eosin stain

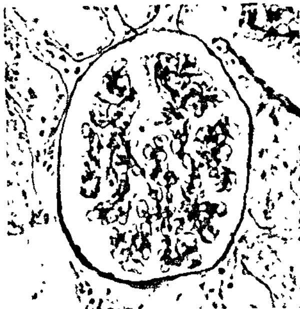


Fig. 41 Glomerulus of normal dog. Periodic acid-Schiff stain.



Fig. 42 Glomerulus of dog; biopsy taken 7 days after last injection of nephrotoxic serum Hematoxylin and eosin stain.



Fig 43 Glomerulus of dog; biopsy taken 7 days after last injection of nephrotoxic serum. Periodic acid-Schiff stain

serum protein values could vary between 4 and 5.3 gm. %, cholesterol between 105 and 165 mg. %, and total lipid values varied from 640 to 960 mg. %. From twelve dogs injected with N.T.S. obtained from rabbits, three died immediately after the injection of the serum, we believe from serum toxicity. Seven of the remaining nine dogs developed renal disease that we believe simulates the nephrotic syndrome. Two of these dogs died before urine or blood chemistry could be obtained. Histologically, Dr. Hackel believes that the glomerular lesions observed looked similar to those previously described in rats The five remaining animals showed proteinuria that varied between 0.5 and 6.0 gm. of protein per twenty-four hours. They showed hypoproteinemia with values as low as 2 6 gm.%, hyperlipemia with cholesterol values as high as 350 mg.%, and total lipids as high as 1300 mg %. Transient hematuria was noted in two dogs and transient hemoglobinuria developed in two animals that were injected with the same serum. This whole project was started not only for the purpose of seeing whether or not one could produce the nephrotic syndrome in another animal, which as such is worth-while knowing, but also with the idea in mind of obtaining biopsies in one and the same animal through the course of the disease Dr. Hackel will show you some of these biopsies later on This project was also gone into in the hope of finding an animal in which ACTH and Cortisone action could be better studied than in the rat in which both of these agents did not prove to be very effective (6). A third and main reason was to go farther ahead in the work on the pathogenesis of the nephrotic hyperlippemia, which the small size of the rat inhibited greatly by yielding only a few cc. of blood or a few gms. of kidney tissue None of the puppies, so far observed, developed edema, but two of them had ascites which in one case was marked. In one dog a moderately severe disease has apparently undergone spontaneous cure That animal became pregnant twice thereafter, and never showed proteinuria In one other dog the disease has lasted now for three months without subsiding. All in all the lesions thus far produced have been mild, possibly some have been moderately severe, but not ever very severe. We have not yet succeeded in producing it in its severest form and we may have to change our immunization and injecting procedures still further. Duck serum has not been used in dogs by us as yet. Blood pressure readings have not been obtained as yet either. Proteinuria was observed without delay, without a latent period noted.

DR HACKEL The following figures illustrate the histological features of the renal disease in these dogs

For purposes of orientation, I will first show these sections of normal kidney stained with hematoxylin and eosin (Fig. 40) and by the periodic acid-Schiff method (Fig 41) The glomeruli contain thin-walled patent capillaries with a fine basement membrane The tubules are normal, with no casts. Figs 42 and 43 are from a dog biopsied 7 days after the last injection of nephrotoxic serum. They show an acute type of response similar to that seen in rats There is marked endothelial swelling and proliferation as well as prominent basement membrane thickening. The capillary lumina are thus largely obliterated and contain very few red blood cells.

Two months after the last injection of nephrotoxic serum, this kidney biopsy shows

(6) Hackel, D G , Portfolio, A G., and T D. Kinney, Experimental Nephrotoxic Nephritis in the Rat Treated with ACTH or Cortisone Proc Soc. Exper. Biol. Med., 74: 458, 1950

a later stage of the same disease (Figs 44, 45). There is still some residual endothelial hyperplasia, but there is also a moderate degree of fibrosis involving the intercapillary tissue and Bowman's capsule.

MEMBER: Did the latter dog still have clinical signs of renal disease?

DR. HACKEL: Yes. At the time the biopsy was taken there was still some hyperlipemia and proteinuria.

MEMBER. How large a piece of kidney did you get for these biopsies?

DR. HACKEL· Roughly, 10 x 5 x 5 mm.

MEMBER: In the disease of these dogs, and also in the rats, has anybody looked at the liver? What does the liver look like?

DR. HACKEL· It looked perfectly normal in the rats

MEMBER· I wondered if there was any kind of a lesion in the liver because there is evidence that part of this antibody material is taken out by the liver, since radioactivity is found there

DR. HACKEL· I have seen no inflammatory lesions in the livers of rats. Very rarely I have seen a small focus of inflammation in the myocardium, but nothing in the liver

MEMBER: But the livers are enlarged

DR. HACKEL: They are enlarged?

MEMBER By weight, they are enlarged, but histologically they do not show anything

DR. HACKEL: It may be attributed to edema

MEMBER: Now that is rather interesting That is why I asked the question about the ascites. I read your paper, and Dr. Pressman's about the antibody studies, and we tried grinding up some liver and also the liver blood vessels, and injecting them back into rats, and they developed very bloody ascites with hypertrophy of the liver to three or four times its usual size, but we couldn't find any histological changes There were no pathological changes in the kidneys. I didn't get beyond that stage. I was never able to explain any of the findings we had. Maybe you can throw some light on it.

MEMBER· Dr Hackel, would you say that the second figure shows the healing stage with scarring? There is no acute inflammation, -- or is there a little acute inflammation there?

DR HACKEL: The biopsy taken two months after onset shows some scarring as well as residual inflammatory response. There is no acute inflammation.

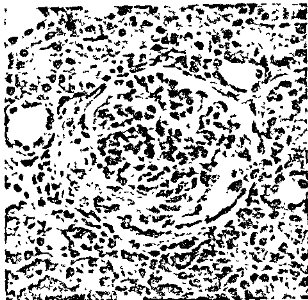


Fig 44 Glomerulus of dog; biopsy taken 2 months after last injection of nephrotoxic serum. Hematoxylin and eosin stain



Fig. 45. Glomerulus of dog; biopsy taken 2 months after last injection of nephrotoxic serum Periodic acid-Schiff stain

DR. LAUSON: Several thoughts occurred to me concerning the technic of doing these experiments. One, biopsy would be made simpler if the kidney were first explanted under the skin. Two, unilateral disease could be produced by injecting a small amount of nephrotoxic serum into the renal artery of the explanted kidney. Three, explantation of the bladder trigone would make it possible to measure functions of the two kidneys separately

DR PRESSMAN: Yes, we've had similar thoughts.

CHAIRMAN RAPOPORT: Dr. Ehrich, will you continue?

DR EHRICH I have been asked by Dr Carolyn Piel and by Dr Modern in California to present their work as well as ours As this is more or less integrated, I shall present it as one story; but I shall point out who did what.

I was very much impressed with the beautiful work that was shown to us yesterday by Dr Hall. You may know that Dr. Modern together with Dr Baker conducted similar studies for a good many years. The concept of the glomerular structure which was presented to this group at previous meetings was based on observations by Baker and Modern.

I do not think that our concept should be changed. Our diagram (Fig 46) shows three layers in the glomerular membrane The outer layer corresponds to the podocytic layer, the intermediate layer represents the lamina densa, that is, the real basement membrane, while the inner layer corresponds to the lamina fenestrata. Thus far we agree with Dr Hall

However, we are not sure that the lamina densa is a product of the capillaries. We believe that it may well be an extension of the intercapillary space, that is, a derivate of the connective tissue which holds the loops together

If Dr Hall does not believe in the intercapillary space, this is due probably to the fact that he worked chiefly with rats. We have been working with rabbits and humans as well The latter species both have fine connective tissue; they are, as you know, good antibody producers, another indication of a strong mesenchyme The rat, on the other hand, is a poor antibody producer; its connective tissue is less conspicuous.

Fig. 47 shows one of the electron microscopic pictures furnished by Dr. Modern It is not as good as Dr. Hall's pictures. But it shows, in a rat glomerulus, what I interpret as an intercapillary space (a)

It is true that Dr Hall would say that this is a tangential section through a basement membrane If I feel that it is an intercapillary space, I do so because we can enlarge it experimentally to a point where its identity is unequivocal. This can be done with horse serum in both rats and rabbits. Samples of these were shown to you two years ago

That human glomeruli, too, possess an intercapillary space is apparent from its

reaction in diabetes mellitus (Kimmelstiel and Wilson's intercapillary glomerular sclerosis). The development of this sclerosis is illustrated in Figs. 48 (early cellular stage), 49 (early fibrous stage) and 50 (late stage).

It was stated yesterday that normal glomeruli do not contain silver or collagen fibers. We find that silver fibrils are easily demonstrated by the Gomori procedure in the glomeruli of rats, rabbits and man, both normal and pathological. For these and other reasons we believe that the glomerulus does contain an intercapillary space, and this functions as a mesangium.

Let me turn now to a second group of studies, namely those which were done during the last three years by Dr. Piel. You may remember that we submitted the thesis that in the rat anti-kidney serum produces either nephritis or nephrosis, depending on the serum injected. If we used a small quantity of serum or a serum of a low toxicity, we got nephritis; if we used a high dose or a strong serum, we got nephrosis.

Dr Piel has now two large series of rats, one treated so that nephritis developed, while nephrosis was induced in the other. In the nephritic animals the serum cholesterol remained practically normal, while in the nephrotic group it was elevated. Similarly, the nephritic animals developed only little proteinuria, while the nephrotic rats showed considerable proteinuria.

MEMBER· Are you defining the difference between those two merely on the basis of the severity of the disease?

DR EHRICH: No, we believe that they are two different diseases

MEMBER· May I point out that this degree of proteinuria in the so-called nephritic one is hardly above the normal proteinuria, which would be here up to 30 mg. per cent that the rat normally has. I would say that this animal has no disease, no proteinuria, normal cholesterol, and had neither nephritis nor nephrosis.

DR. EHRICH: Dr. Piel relates that the nephritic group developed protein levels in their urine from 10 to 69 mgm.%/24 hrs. The average value was 34 mgm.%. It was greatest in the 2-4th week of the experiment. It diminished after 4 weeks. The nephrotic group, on the other hand, showed values from 15 to 634 mgm.% with an average of 211 mgm.%. This was severe in the first weeks of the disease, but became more pronounced as the disease progressed in animals severely affected. The urinary protein of normal rats varied from an average of 4 mgm.% in animals weighing 100 grams to 62 mgm.% in animals weighing 400 grams. The rats used in the present experiment weighed 50 grams. At this weight no proteinuria was detected per 24 hours.

The nephritis was characterized morphologically by swelling and proliferation of endothelial cells. This was most marked in the first two weeks, then it receded, and after 4-8 weeks it was practically gone. In other words, the proliferative nephritis came on rapidly in a week and subsided in a month or two, that is, very much like in humans.

In the nephrotic group, we found very little proliferation, if any, although slight

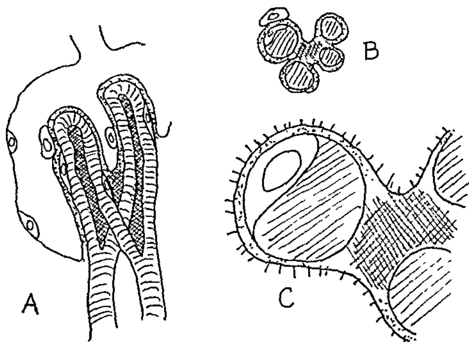


Fig 46 Diagram showing the three layers of the filtering membrane and the intercapillary space of a glomerulus in a longitudinal section (A) in a cross section (B) and in the electron microscope (C)



Fig 47 Section of normal rat glomerulus showing eight capillary cross sections Six of these are united by a dark material some of which may represent tangentially cut filtering membrane The heavy material in the centre is interpreted as intercapillary tissue Note the podocytes sur-

degrees were observed occasionally. The outstanding change in these animals was thickening of the basement membrane as described two years ago. The latter change was inconspicuous in the nephritic rats, although some was seen in some rats.

Hematuria, also, was observed in the nephritic group. This is well illustrated in Fig. 51.

MEMBER: How did you gauge these things?

DR. EHRICH: The hematuria was gauged in two ways. Microscopically, and by urinalysis.

MEMBER: Excuse me, but do these rats represent one rat or a mean of many rats?

DR EHRICH. An average of about 100 rats.

Let me turn now to the progress of the rat disease. You may remember that we distinguish between pure renal diseases, such as pure lipid nephrosis characterized by a thickening of the basement membrane, pure glomerular nephritis distinguished by inflammatory infiltration and endothelial proliferation, and pure diabetic and lupus nephrosis. Most of these conditions have a tendency to heal In other cases, the pure disease may be complicated by thrombosis of glomerular capillaries and exudation into the capsular space followed by scar and crescents formation It is the latter group which succumbs and dies in renal failure. Evidence to this effect has been shown to you in a previous session.

Dr Piel has followed her animals for 2-3 months after injection of serum. It was found that of the nephrotic animals about 50% developed serious complications causing renal insufficiency This figure corresponds very closely to that in children suffering from lipid nephrosis. On the other hand, of the nephritic animals only a few developed severe disease which is in keeping again with our experience in man.

Many diseased rats were biopsied by Dr. Piel 1/2, 1, 2, 3, 4 or 5 weeks after injection of the serum All of these were sacrificed after 8 weeks Surprisingly it was observed that the biopsy procedure aggravated the rat disease. It was found that biopsied animals developed a higher incidence of severe disease than non-biopsied controls. Many animals with a mild nephrosis at biopsy revealed severe disease at autopsy Moreover, the earlier the biopsy, the more detrimental the procedure

The mechanism of this aggravation appeared to be one of precipitating thrombosis in glomerular loops. Glomerular thrombosis followed by death in uremia was observed even in normal rats subjected to biopsy Hence, it appears that the biopsy procedure is not an innocuous procedure in the rat.

MEMBER: You mean a biopsy of one kidney will precipitate that.

DR. EHRICH The pieces of tissue taken from the kidney were very small. They contained not more than about 100 glomeruli

MEMBER: But the other kidney got diseased from the biopsy of the one kidney?

DR. EHRICH: Both kidneys were equally involved.

Microscopic studies of the biopsy and autopsy material submitted by Dr. Piel revealed essentially the same changes which we described at earlier sessions.

In the nephritic rats we observed not only swelling of glomerular cells, but also considerable increase in the number of nuclei. Electron microscopic pictures showed that these nuclei were within the capillaries, that is to say, the nephritis was intracapillary in nature. The increase in the number of nuclei was variable. In some they were increased about 100%.

In nephrosis we found thickening of the basement membrane with enlargement of the epithelial cells. The number of the nuclei was not increased. Studies with the electron microscope showed deposits at the inner surface of the lamina densa (Fig. 52). It may well be that these deposits were responsible for the thickening of the basement membrane as seen in the light microscope.

The progress of the nephrosis varied with the severity of the disease. While mild cases showed but a few scars when sacrificed, severe cases developed progressive thrombosis of glomerular loops with exudation into the capsular space and subsequent scar formation and obliteration. The exudate in the capsular space could be traced to glomerular loops which were obstructed by thrombi.

In animals sacrificed after two weeks or later old thrombi and capsular exudate replaced by collagenous scars and crescents were commonly found side by side with fresh thrombi and fresh exudate containing fibrin (Fig. 53). This was interpreted to mean that the disease started by one injection of serum was progressive.

MEMBER: How do you know that was due to the thrombosis?

DR. EHRICH: Because we have numerous slides both of biopsies and autopsies which clearly show this relationship. Fibrin or fibrinoid is laid down continually. This observation supports our thesis that it is this complication which causes more and more obstruction of glomerular filtration and eventually uremia. The same complication which occurs in lipid nephrosis is seen also in other nephroses, such as lupus nephrosis and in diabetes. It occurs also in some cases of glomerular nephritis. We believe that this complication is a common cause of renal failure in these various diseases.

We have learned then that the experimental rat nephrosis which has been studied so well by Drs. Heymann and Hackel, is not a limited reaction to an initial insult, but it is a chain reaction which continues for weeks and months. The cause of this progress has not been elucidated, but I believe that immunological studies might furnish the answer.

The rat nephritis differed from the nephrosis in that it appeared to be self-limiting; it healed in about 90% of our animals within four weeks.



Fig 48 Cellular proliferation in the intercapillary space in an early case of intercapillary glomerular sclerosis.



Fig. 49 Collagen deposition in the intercapillary space in a more advanced case of intercapillary glomerular sclerosis.



Fig. 50. Heavy collagen deposition in the intercapillary space in a late case of intercapillary glomerular sclerosis.



Fig. 51. Hemorrhage into a glomerulus and tubule in a rat with experimenta glomerular nephritis.



Fig. 52. Section of a loop of a nephrotic rat glomerulus showing partial thickening of the filtering membrane due to a deposit in, or on the inner surface of, this membrane Note the vesiculation of the podocytes surrounding the loop. Magnification approximately x 7000

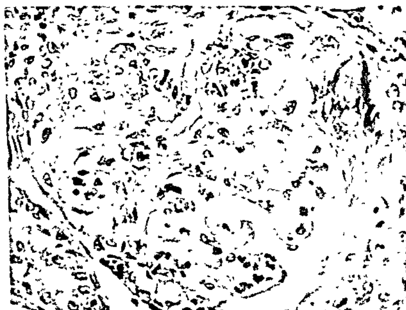


Fig. 53. Old fibrosis of glomerular loops (at top of picture) and recent deposition of a fibrinous material in the capsular space (at right) of a rat with lipid nephrosis of two months duration.

We learned also that in rats biopsy of a kidney may aggravate the disease.

Let me turn now to a group of therapeutic studies which were conducted in association with Drs. Seifter and Sharma.

I have stressed our belief that the deposition of fibrin or fibrinoid in the glomerulus plays an important role in the progress of the disease and its fatal outcome both in animals and patients. If this is correct anticoagulants, such as heparin or paritol, or fibrinolytic or proteolytic enzymes, such as fibrinolysin or trypsin, should have a beneficial effect.

We have studied so far the effect upon acute lipid nephrosis in rats of heparin and paritol We also used hyaluronidase, collagenase and cortisone.

MEMBER: What is paritol?

DR. EHRICH: Paritol is a synthetic Heparin which differs from native Heparin in that it is not an amino-sugar.

First I should like to point out that we produced a nephrosis which killed 70% of the experimental rats within a week. We thought that a severe disease would lend itself to therapeutic experiments better than a mild disease.

Table 8

	Dead in 7 Days %
Serum Control	70
Serum +Hydase	75
Serum + Collagenase	90
Serum + Cortisone	80
Serum + Heparin	42
Serum + Paritol	58

Death rate of rats with severe nephrosis treated with 1) five subcutaneous injections of 10,000 T.R.U. each of hyaluronidase on 5 successive days beginning 2 days before injection of serum; 2) one half mg. each of collagenase injected as in 1; 3) five intramuscular injections of 2.5 mg. each of cortisone given as in 1; 4) two intravenous injections of 5 mg. per kg. each of heparin 10 minutes before and 1 hour after injection of serum; 5) 15 mg. per kg. each of paritol given as in 4.

Table 8 shows that heparin and paritol in contrast to hyaluronidase, collagenase and cortisone, depressed the death rate. Of the heparin treated rats only 42% were dead in seven days, that is, only half the number of the control groups.

MEMBER: Were the final mortality figures on those you have a 7-day limit -- what was the total mortality rate?

DR. EHRICH: We conducted only short-term experiments of one week's duration. We used more than 100 animals which is all that we could manage at this time. We plan to conduct long-term experiments later.

Tables 9 and 10 show the effect of our treatment upon the edema caused by the disease. This was determined in two ways: 1, by studying the weight increase of the animals, and 2, by estimating the quantity of ascitic fluid *found in their abdomen.* Edema was felt to be present when the total weight of the rat increased 15% or more over the normal weight increase.

Table 9

Incidence of edema as measured by weight increase in the various experimental animals which survived 4 days or longer.

	Weight increase of 15%		
Serum ml.	0.2	0.3	0.5
Serum Control	2 of 4	3 of 3	2 of 3
Serum + Hydase	1 of 2	1 of 1	3 of 3
Serum + Collagenase	1 of 1	0 of 1	1 of 1
Serum + Cortisone	1 of 2	0 of 2	--
Serum + Heparin	0 of 4	0 of 3	0 of 4
Serum + Paritol	0 of 4	0 of 3	1 of 2

Table 9 shows that of the animals who received heparin or paritol and who survived 4 days or longer, only 5% developed a weight increase of 15% or more over normal, while in the control groups this figure was 65%

MEMBER: Was the food intake the same? Did you measure it?

DR. EHRICH: Yes, they were treated completely alike.

Table 10 shows the effect of our treatment upon the ascites caused by the disease. It was found that this is a better measure of edema than the weight increase. Apparently ascites develops first, *maybe because the belly of the rat is its depending part.* I believe Dr. Heymann will concur

It can be seen in the table that in the few animals treated with heparin or paritol who died during the first 4 days ascites was as common as in the various control groups. During the 5 to 7 day period, however, ascites was found in 80% of the controls,

but in only 40% of the rats treated with heparin or paritol. These various figures show clearly that the edema of our disease may be depressed by anticoagulants.

Table 10

Incidence of edema as measured by presence of ascites. The hyaluronic acid was prepared from streptococci. It was given intraperitoneally in doses of 100 mg. each twice daily beginning 2 days before injection of serum.

	Ascites	
Days	1-4	5-7
Serum Control	11 of 12	6 of 10
Serum + Hydase	13 of 17	4 of 6
Serum + Hyaluronic acid	6 of 9	----
Serum + Collagenase	3 of 3	2 of 3
Serum + Cortisone	3 of 4	3 of 4
Serum + Heparin	0 of 1	4 of 11
Serum + Paritol	3 of 3	4 of 9

Table 11

Incidence of severe renal disease in rats who survived the 7 day period of the experiment

	Severe Renal Disease Microscopically	
Serum Control	2 of 3	
Serum + Hydase	3 of 3	86%
Serum + Collagenase	1 of 1	
Serum + Cortisone	1 of 3	33%
Serum + Heparin	3 of 7	33%
Serum + Paritol	1 of 5	

Table 11 reveals that the severity of the disease as seen in the microscope is likewise depressed by heparin or paritol. Severe renal disease was found in 80% of the hyaluronidase or collagenase treated or the untreated rats who survived 7 days. It was present in only 33% of the heparin or paritol treated animals. It is noteworthy that cortisone had a similar effect.

Tables 8-11 show that hyaluronidase had no effect on the death rate, the edema or the microscopic appearance of the disease, but it depressed the blood cholesterol. Hyaluronic acid had a similar effect (Table 12). This is not an isolated finding. Similar results were obtained in hypercholesterolemia in experimental rabbit atheromatosis, and also in the Shwartzman phenomenon (1). We believe that this is due to a detergent action upon the chyolesterol of hyaluronic acid, or of mucopolysaccharide liberated by hyaluronidase.

Table 12

Cholesterol levels of normal and nephrotic rats, untreated and treated with hyaluronidase or hyaluronic acid, 3 days after injection of serum.

Total Blood Cholesterol (mg. %)

	Saline Control	Serum Control	Serum + Hydase	Serum + Hyaluronic acid
	91	171	99	120
	114	168	96	96
	140	236	73	104
	112	215	110	81
	165	248	101	92
	126	181	90	87
		248	98	90
		301	100	101
		241		
		331		
		301		
Mean	125	240	96	96

MEMBER: Did they all get the same sera and the same dose?

DR. EHRICH: Yes, all received one single injection of the same serum.

MEMBER: What was the dose of Heparin?

(1) Seifter, J., Baeder, D.H., Beckfield, W.J. Sharma, G.P., and Ehrich, W.E., Proc. Soc. Exp. Biol. a. Med. 83: 468, 1953.

DR. EHRICH The dose of Heparin that was given in this particular experiment consisted of 2 intravenous injections of 5 mg. per kg. each, 10 minutes before and one hour after injection of serum. In a recent experiment which has not yet been evaluated 25 mg. of Heparin were given every day. Some of these animals developed hemorrhagic ascites, and their death rate was not depressed. Obviously, they received too much Heparin.

MEMBER: Were those cholesterol levels done on the same animal that the ascites measurements or the weight measurements were done on?

DR. EHRICH. Ascites and weight increase like histological changes were studied in all animals, while cholesterol was determined only in the group that was shown in Table 12.

MEMBER: It was not the same group as the previous tables?

DR. EHRICH In part. The cholesterol animals were included also in Table 10, the only other table containing animals dead or sacrificed during the first 4 days of the experiment.

MEMBER What type of Heparin was that?

DR. EHRICH: Lederle's Heparin.

MEMBER We have used Heparin and Paritol-C in rats, not initially, but during later stages of the disease without seeing anything, as far as the course of the disease goes, or as far as proteinuria was concerned

DR EHRICH This may be explained by the fact that fibrin is collagenized rapidly Heparin and paritol prevent fibrin formation but they do not dissolve collagen. However, they may stop the progress of the disease which is due at least in part, as was demonstrated, to continued precipitation of fibrin or similar materials. We know for sure that anticoagulants interfere with thrombosis. It has been shown, for instance, that the generalized Shwartzman reaction in the kidneys may be prevented by this mechanism

DR. LANGE It practically makes the complement inactive, and thereby the Shwartzman phenomenon. Certain dangerous conclusions might be drawn from these experiments These studies, as Dr. Heymann has so beautifully shown just before, are a one-term immunological event. These rats get a complement drop of antigen-antibody lasting for 24 hours, probably less than that. Since no scarring occurs, therefore probably some of these experiments cannot work, theoretically.

DR LANGE: But all these substances studied act on the antibody formation. And if there's no antibody formation going on any more, the whole thing cannot work.

DR. EHRICH I am sorry that I cannot concur in this view. It is well established that cortisone and similar materials do not act merely by suppressing antibody formation. In fact, this seems to be a mechanism only with very large doses of cortisone,

doses larger than those given in humans. But it acts by its so-called antiphlogistic action. This was well illuminated at the M and R Symposium on Immunity and Allergy at San Francisco last week.

MEMBER: What is that -- "antiphlogistic action"?

DR. EHRICH: The antiphlogistic action of the adrenal steroids is very complicated. It involves the fibroblast, the metabolism of hyaluronic acid, the production of collagen, the removal of micro-organisms. Also it affects neutrophiles, plasma cells, and eosinophiles. It is very complicated indeed.

MEMBER: If it were an antiphlogistic action, it should occur immediately, but it does not occur immediately; it occurs after ten days.

DR. EHRICH: The other point on which I am at variance with you concerns the antigen-antibody reaction in the experimental renal disease. I do not believe that this is a one-term immunological event. At San Francisco the conclusion was reached that the initial antigen-antibody reaction causes the production of tissue antigens and auto-antibodies, and that this reaction may continue some time.

MEMBER: Is anything known about the clearance of Heparin?

DR. EHRICH: Heparin has been studied very nicely. It is excreted chiefly through the kidney. During this process it is concentrated in the proximal convolution. It is also reabsorbed here. Its presence can be demonstrated histochemically in both the epithelium of the proximal convolution and in the lymph vessels going out of the kidneys. Hence it may be looked upon as a target drug for kidneys.

MEMBER: How long would you want to give a human case of nephritis Heparin?

DR. EHRICH: I could not answer that. So far we have studied only the first 7 days of the experimental disease.

CHAIRMAN RAPOPORT: That is, as nephrosis -- I don't think any of us would want to do anything in the case of nephritis.

MEMBER: I spoke with Dr. Irving Wright about the possibility of using Heparin - and he knows a good deal about its use, clinically. He said that he would not do it, that is, that he would not be responsible for us doing it. He suggested we try other antigoagulants, that he thought Heparin would be too dangerous to use in any child with anything.

DR. HACKEL: Dr. Heymann and I disagree with Dr. Ehrich's interpretation of the effects of small and large doses of nephrotoxic serum in rats -- that small doses result in the picture of "glomerular nephritis" and that large doses result in "nephrosis." We feel that the same renal disease is produced independent of the dose, and that this disease more closely resembles human "nephrosis" than "nephritis." The only difference is that small doses produce a mild disease and large doses a severe disease. Histologically, too, there is only a quantitative difference, so that both basement membrane swelling and cellular proliferation may occur in varying proportions in different

rats given the same dose of nephrotoxic serum. We have previously reported a series of rats (2), each being injected intravenously with one large, potent dose of the same batch of nephrotoxic serum. They were killed after the injection at intervals of 1/2, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours.

A glomerulus from a rat killed only one hour after a single injection of nephrotoxic serum is illustrated in Fig. 54. There is a marked hyperplastic reaction, with diffuse exudation of polymorphonuclear neutrophils and obliteration of the capillary spaces. At the same time the periodic acid-Schiff stain demonstrates prominent basement membrane thickening. Fig. 55 is a section of a glomerulus from a normal control rat injected with blank rabbit serum. The thin, patent capillaries of the glomerular tuft contrast with those shown in Fig. 54.

After 24 hours the glomeruli still showed some hyperplastic response and endothelial swelling It was not as marked and the capillary lumens were not completely obliterated. The basement membrane showed even greater thickening at this time, as demonstrated by the periodic acid-Schiff stain.

After 72 hours the changes were similar to those after 24 hours, but the hyperplastic component was less prominent while the basement membrane thickening was more pronounced.

The histological picture after 1 hour is thus indistinguishable from some cases which Dr. Ehrich has called "nephritis", whereas the picture after 24 hours is like his "nephrosis" Both were produced by the same dose of potent nephrotoxic serum; and the animals developed the urinary and blood chemistry changes that are typical of nephrosis

DR HEYMANN Dr Ehrich, do you still say that some sera produce nephritis and others produce nephrosis?

DR EHRICH Yes.

DR HEYMANN· And if you double or triple the dose of the typically nephritis producing serum it still will produce only nephritis?

DR EHRICH· We do not have too much experience with different doses of one and the same serum although we have some. In these rats small doses produced what we call nephritis, while large doses of the same serum caused nephrosis The experiments which were reported today were carried out with different sera.

DR. HEYMANN· We know that very potent large doses of the serum that produce clinically and histologically various stages of disease histologically produce typically nephritic lesions

(2) Heymann, W and Hackel, D.B. The Early Development of Anatomic and Blood Chemistry Changes in Nephrotoxic Renal Disease in Rats. J. Lab. & Clin. Med. 38·1 (1952)

DR EHRICH· The photos of the lesions which were shown by Dr. Hackel were not too clear, but I would call all of them nephrosis.

DR. HEYMANN: Even the nephritic histology?

DR. EHRICH· I did not see any nephritic histology. It is true that during the first 24 hours we always find a few leucocytes in the glomeruli, but that is not glomerular nephritis. There are many differences between the two diseases. The nephritic group of animals tends to recover in a month. It shows hematuria both in the urine and in the kidneys, as I showed you.

DR. HEYMANN No, that is not correct In our animals they do not develop hematuria with small doses. We have some with the severe disease that do develop hematuria, and we do not believe that this hematuria is diagnostic of either the nephritic or nephrotic situation.

DR EHRICH· We speak of nephrosis when the disease is associated with marked proteinuria, hypoproteinemia, hypercholesterolemia and edema, clinically, and with thickening of the basement membrane, histologically.

DR. HEYMAN· Oh, no.

DR. EHRICH *I spoke of the nephrotic syndrome. We do not think that these* diseases are etiologically different. However, we do believe that this etiologically uniform disease takes two different courses. In other words, they are pathogenetically different.

DR. HEYMANN Mild and severe.

DR. EHRICH: Not merely mild and severe. It is true that glomerular nephritis is the milder disease, both in the rat and in humans. But there is a difference in the antigen-antibody reactions in these two cases. The nephrosis comes on at once, the nephritis after a delay period. It is generally agreed that the rabbit disease caused by anti-kidney serum is essentially nephritis. It is entirely possible that the quality of the duck serum conventionally used in these experiments may have something to do with it. But I still believe that quantity is a factor. Rabbits can be given only 2-4 cc of serum per kilo; if you give more you will kill the rabbit. On the other hand, rats can be given 30 cc or more, and it is this large dose which is given usually to produce nephrosis.

MEMBER: Would it be possible to titer the antitoxin level by complement fixation test and thereby quantitate the actual amount of material?

DR EHRICH· I think it most desirable that such work be done.

MEMBER: No, but the activity of the serum and its titer in the kidney of the recipient species to which it is given had no relation whatever.

MEMBER Various people have agreed with that, that there is no relationship.

DR. RAPOPORT: *Dr. Bessman will present her data next.*

DR. BESSMAN: I would like first to comment on the remarks of Dr. Ehrich and Drs. Heymann and Hackel regarding the production of nephrosis or nephritis when one uses a weak or a potent serum. Our results have agreed with the latter two workers, i.e. whether a weak or potent serum is used, the nephrotic type of picture is produced. The severity of the disease depends on the potency of the serum. All of our rats were 18-21 days at the time of injection, and were Sprague-Dawleys. Maybe the age and strain are factors.

Our nephrotic rats were prepared according to the technique described by Heymann (3), using nephrotoxic serum obtained from rabbits. I was working with Dr. Talbot, and we were also interested in the problem raised by Dr. Rapoport yesterday, of whether different salt intakes have any specific effect on the course of the nephrotic syndrome, but as observed in rats. We controlled all other factors as far as possible, changing only the salt intake of the various groups. All rats were the same sex, initially the same weight, given the same dose of the same nephrotoxic or bland rabbit serum and the same diet Saline was added as a liquid to the standard diet. Controls were run at each salt intake level, and were pair fed with the nephrotic animals. We were studying electrolyte balances at the same time so unfortunately there are only six rats in each diet group; three nephrotic and three controls

The four levels of salt intake were as follows: 1 1.5 milli-equivalents (meq.) Na/sq meter/day, or a diet roughly comparable to a rice diet in humans; 2: 15 meq. Na/sq. meter/day, or a "low" sodium diet, 3: 70 meq. Na/sq. meter/day, or a low normal diet; and 4: 150 meq. Na/sq. meter/day or a normal salt intake The absolute amounts of sodium ingested on the four above diets were 0.03, 0.3, 1.4 and 3.0 meq. Na per rat per day respectively. Incidentally, we analyzed the standard Sprague-Dawley diet and found that it contains 0.3 meq. Na per gram. Since our average rat ate 10 grams of food per day, our average rat would have had an intake of 3.0 meq. of Na per day on that diet. This amount is the same as our highest sodium diet. In other words, our highest ration of sodium is the equivalent of a normal optimal intake for these rats So none of our diets were really "high" sodium diets in the sense of being at a toxic level to normal rats, but on the contrary contained no higher than that amount present in standard rat diets.

All rats were killed six weeks after injection of serum (unless death intervened) and Table 13 presents a summary of much of our data. The serum values are on blood taken at the end of the six week period. The urine values are averages of bi-weekly measurements.

As can be seen from this table, the mortality rate of all controls and of the nephrotic rats on the two lower sodium intakes was zero. Of the six rats on the higher sodium intakes, three died; i.e. a 50% mortality rate. One of these was on the 70 meq /sq. meter sodium intake, and the other two were on the 150 meq./sq. meter intake.

(3) Heymann, W. and Lund, H Z: The Nephrotic Syndrome in Rats, Pediatrics 7:691, 1951

TABLE 13 - DATA ON NEPHROTIC AND CONTROL RATS ON VARYING SALT INTAKES AT THE END OF A 6 WEEK PERIOD

SALT INTAKE (SODIUM)

	150 meq / sq.meter/day (3 0 meq / day)		70 meq / sq.meter/day (1.4 meq / day)		15 meq./sq meter/day (0 3 meq / day)		1 5 meq./sq.meter/day (0.03 meq./day)	
	Control	Nephrotic	Control	Nephrotic	Control	Nephrotic	Control	Nephrotic
MORTALITY	0 of 3	2 of 3	0 of 3	1 of 3	0 of 3	0 of 3	0 of 3	0 of 3
SERUM LIPIDS	333	628*	299	688**	300	570	300	941
SERUM SODIUM (meq /1)	150	146*	147	146**	142	149	141	160
SERUM POTASSIUM (meq./1)	5.5	4 7*	4 3	5.2**	4 3	4 7	4 5	4.6
SERUM CHLORIDE (meq./1)	105	105*	109	110**	99	91	104	103
SERUM PROTEIN (gms./100 cc)	6 2	6.0*	6.1	6.1**	5 9	6.2	6 0	6.5
SERUM BUN (mgm./100 cc)	19	21*	22	29**	28	23	27	42
Daily average over a six week period PROTEINURIA (mgm./day)	<5	300	<5	339	<5	87	<5	85
URINE VOLUME	20	18	16	22	18	9	27	12

* only one rat
** only two rats
Serum lipids measured in cubic millimeters.

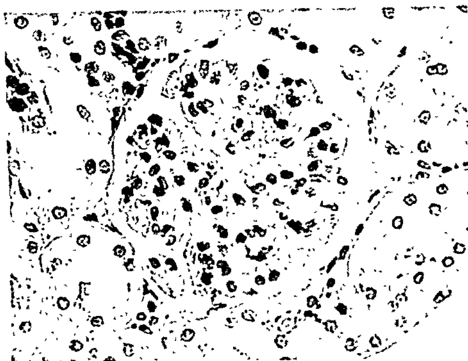


Fig. 54. Normal glomerulus from rat injected with blank rabbit serum.

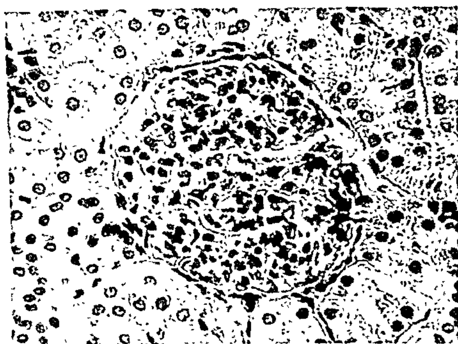


Fig 55 Kidney from rat killed 1 hour after a single intravenous injection of nephrotoxic serum. Hematoxylin and eosin.

Two of the three rats that died had massive ascites and pleural effusions. These mechanical factors may have contributed substantially to the demise of these rats. However, the third rat which died had no edema at the time of death. This animal had had ascites during the first week of the disease, lost the edema, and subsequently died on the 15th day of the disease.

Total serum lipids in all control rats ranged between 250 and 350 mg%. The figures for the nephrotic animals can be seen in the table. It is clear that the rats on a severely restricted sodium diet (1.5 meq. sodium/sq meter/day show the highest serum lipid level (941) although all of the nephrotic animals showed elevated levels at the end of six weeks. It is of interest to note that the total serum lipid level is the only consistently abnormal chemical finding at this stage of the disease. If hyperlipemia is an essential component of the nephrotic syndrome, this relationship of electrolyte metabolism to lipid metabolism is very interesting.

You might say that the serum lipids were elevated on the basis of dehydration of those rats severely restricted in sodium intake However, the serum sodium levels in these animals were elevated only 7% above the controls and the other nephrotic rats, while the serum lipid elevation was over 30%

Other than the severely salt restricted nephrotic rats mentioned above, all serum sodium values were within normal limits Serum potassium, chloride, and protein levels were also essentially normal in all groups, i.e all animals which survived the six week experimental period This was true of the blood urea nitrogen (BUN) also, with the exception again of the severely sodium restricted nephrotic animals. These rats showed an elevated BUN of 42 mgm.% as compared to a BUN of 27 mgm.% of their pair fed controls

The average proteinuria is presented in Table 13 None of the controls ever excreted over 9 mgm./day, and the average was less than 5 mgm./day. The rats on the two more liberal salt intakes can be seen to have tremendously increased proteinuria (306 and 339 mgm /day) over those on the low salt diets (87 and 85 mgm./day). In the nephrotic rats there is a concomitant increase in urine volume with increased salt consumption, but not of the same magnitude as the increased proteinuria. Thus the increased proteinuria of the higher salt intake rats may be due to two factors, an increased urine volume and a more severe disease. For reasons unknown to me, the normal rats on the severely restricted sodium diet showed an increased urine volume.

Table 14 presents data on the overall weight changes The rat that survived the normal salt intake (3 0 meq. Na/day achieved a final weight (6 week period) equal to his pair fed controls That the other nephrotic animals achieved a less satisfactory final weight as compared to their controls can be seen from the table The nephrotic rats on the low and low normal sodium diets were 19% under the weight of their controls The most striking lack of growth was seen in those animals which received the severely restricted sodium diets The growth of the control rats was retarded as might be expected But the nephrotic animals on this diet gained only forty-seven grams in six weeks, achieving a final weight which was only 66% of the final weight of the controls In general, the rate of growth is most severely retarded in the first week or two of the disease After that, the growth rate picks up; i.e. during the convalescent

phase growth returns to or toward normal. This phenomenon is likewise seen in children with this disease. The presence of ascites complicates measuring growth by weight gain but tail length is also a good criterion of growth, in the rat.

It appears that the mechanism for maintaining electrolyte (chiefly sodium) homeostasis is deranged in the nephrotic syndrome. If too little salt is given, very poor growth, slight dehydration, elevated serum lipids and BUN's ensue. Too much salt (in this case, an "average" diet) results in edema accumulation, increased mortality, and increased proteinuria. From our limited data, it appears that the low sodium diet (15 meq. Na/sq.meter/day) is the diet of choice from the electrolyte point of view. I have nothing to say on the low or high protein diets controversy.

The rats that were prepared for Dr. Keitel were prepared in the same manner as our other rats. One group received bland normal rabbit serum (controls), one group weak nephrotoxic serum (mild disease) and one group potent nephrotoxic serum (severe disease). Table 15 shows the clinical and laboratory findings in these rats. The normal and mildly ill rats were killed 11 days after their first injection; the severely ill animals were killed 6 days after their first injection. Dr. Keitel will present the data on the tissue analyses.

MEMBER: Was the potassium intake the same in these diets?

DR. BESSMAN: The potassium was the same.

MEMBER: Did they eat about the same feed, approximately?

DR. BESSMAN: They were kept at the same intake. Naturally the ones on the higher salt diet would have eaten more if they had been given more.

MEMBER: How much of the final weight was edema, do you have any idea?

Table 14

GROWTH OF NEPHROTIC AND CONTROL RATS ON VARYING SODIUM INTAKES

Sodium Intake								
Per sq meter	1.5 meq /day		15 meq/day		70 meq/day		150 meq /day	
Total amount	0.03 "		0.3 "		1.4 "		3.0 "	
	Control	Nephrotic	Control	Nephrotic	Control	Nephrotic	Control	Nephrotic
Initial weight (grams)	58	50	55	54	58	53	54	48
Final Weight	146	97	184	155	182	145	190	179
Weight Gain	88	47	129	101	124	92	136	131

Table 15

	Normal	Mildly Nephrotic	Severely Nephrotic
Serum Protein	6.0 gms.%	5.5 gms.%	4.7 gms.%
Serum BUN	25 mgm.%	38 mgm.%	42 mgm.%
Serum lipemia	absent	absent	present
Ascites	absent	questionable	three plus
Proteinuria	absent	two plus	four plus
Casts in urine	absent	rare	numerous

DR. BESSMAN: Those rats which developed edema either died or subsequently lost it. I would say there was no edema at the end of six weeks. None of the rats on the severely restricted sodium diets developed any ascites or edema. Those on the low salt diet developed transient one to two plus ascites, those on the 70 and 150 meq. sodium/sq. meter/day diets developed three to four plus edema and ascites. By the end of the fourth week all of the ascites had disappeared. Of course, none of the controls developed any ascites.

DR. KEITEL: I will present studies showing body composition in animals with this syndrome described by Dr. Bessman. The first set of data are on skeletal muscles, heart, liver, and kidney. The water contents of the tissues of the control rats, the mildly ill nephrotic rats, and the moderately ill edematous rats, was identical.

The potassium content in the skeletal muscle of control rats was 98; of the mildly ill rats, 95; and the moderately severely ill rats was 91. Heart showed a similar type of descending potassium content of 76, 73, and 66 millimols of potassium. But in the case of the liver and kidney, there was no change in the potassium content in the three groups of rats.

The red cell potassium content in millimols potassium per kg. of red cell was higher in the ill nephrotic rats, perhaps due to the acidosis although we do not have the pH measurements. In other words the red cells seem to behave like the liver or the kidney, in this instance there was a suggestion of potassium deficiency. The total potassium content in millimols of potassium per hundred grams of fat-free solids showed a decrease of about 20%. We measured the length of tail in the three groups of rats, they were sick only for a week, and of course you could hardly expect to find a difference, or if there was, you couldn't measure it by this method. The blood was obtained on these rats by shaving the hair from the neck, and then doing a quick guillotine procedure. The blood was guided into a test tube by way of a funnel. The purpose of this method was to know available blood volume. The concentration of minerals and water per kg. of plasma: sodium was 139 in the control rats, 138 in the mildly ill ones, and 129 in the severely ill, and the chlorides were 103, 104, 114;

CO_2, 26, 24 and 15. We did not get pH measurements. The water content in gms. of water per kg. of plasma in the control rats was 915 gms. per kg. of plasma, in the mildly nephrotic 915, and in the moderately ill rats was 925. And the osmolarity of the serum as measured by freezing point depression in these 3 groups of rats was 310, 308, and 292 milliosmols per liter in plasma.

DR. METCOFF: In regard to the studies of Dr. Keitel, while the data are most intriguing, it is important to remember that the data were for wet non de-fatted muscle. This is particularly important since it was shown that the total muscle mass had decreased; however, with the increase of edema in the more severely affected animal, it is rather interesting that total muscle water did not change.

CHAIRMAN RAPOPORT: Dr Holliday will now present some interesting observations on the results of electrolyte deficiencies in rats.

DR HOLLIDAY: Most of you, or all of you, I am sure, are familiar with the syndrome of potassium deficiency The only reference I will make to it is to point out what I think are more or less the important aspects of it as they relate here They are of course the elevated serum bicarbonate, the low muscle potassium, the high muscle sodium a renal lesion in the tubule, which was originally described by Dr. Darrow as hypertrophy and hyperplasia, presumably on the basis of initial destruction, but this is uncertain yet, and apparently without edema.

Dr Darrow in his original paper described removing chloride from the rat by peritoneal dialysis and getting at least this same picture We have for the past year been engaged in a rather detailed study of that preparation, removing chloride from the animal by peritoneal dialysis. We were primarily interested in the composition of the urine, but that data is not pertinent here. We did find, though, that within 3 days after you removed chloride you got a very intense renal lesion apparently in the proximal tubule We had severe calcification, marked myositis, and apparently a very active process going on

Now a word about how we prepared these animals because it will be pertinent to some remarks following

In order to better evaluate their electrolyte excretion, all of these animals were placed for 3 days on an electrolyte-free diet by the dietitian. The sodium, potassium, and chloride were removed and the diet had a very low calcium and phosphorous content. At the end of 3 days they were excreting essentially electrolyte-free urine, or at least with respect to those ions When we removed the chloride, we had several groups treated differently beyond that point In one group we did nothing but remove chloride, and they had this renal lesion with a moderate degree of intensity

Another group we gave orally, sodium bicarbonate solution, added to their drinking water, and they had a much greater intensity of renal lesion, and I might say they had a much greater reduction of muscle potassium, and much higher muscle sodium.

Then we gave another group sodium acid phosphate in the same molar concentration and they had the most intense changes of all their bicarbonate was not so high as those fed the sodium bicarbonate but their muscle changes were more severe, and their renal lesions were apparently more severe We don't have enough yet to make real, definitive, comparative statements.

We also had a group that instead of just giving sodium bicarbonate, we gave a mixture of sodium and potassium bicarbonate. That was to see if among other things it would have any effect on the potassium concentrations themselves and actually, as a side line, what it might do to this renal lesion. It did not have any effect upon the concentrations of potassium themselves and the renal lesions develop just as they did with sodium bicarbonate, alone.

We made them alkalotic and we know they developed the K deficiency in the 3-day period ensuing. We started attacking this prior to making them alkalotic. However,

there is a gimmick to this and that is the effect of the electrolyte-free diet on rat muscle composition -- we talked about this last year at Atlantic City -- so some of you are familiar with it. But within the 3-day period on the electrolyte-free diet nothing happens to their serum bicarbonate. Nothing happens to their muscle sodium, and nothing happens, as far as you can see, to the rat. They clinically appear well, but their muscle potassium does drop from around 47 millimols per 100 gms. of fat-free tissue to about 41. So we have to measure our potassium deficiency due to alkalosis from that point on, and then it goes on down quite a bit from there.

Now the rats on the electrolyte-free diet had no alkalosis, and at that point had no increase in cell sodium, and no renal lesions. Now in a sense this terminates one concept, and the only one I intended talking about, and that is the renal lesions in association with alkalosis, and which of these three variables it was more or less dependent upon. I'm still not sure -- the alkalotic rats that we prepared obviously are not edematous since we have taken chloride away from them, and they could not possibly become edematous if they wanted to.

MEMBER: Why not?

DR. HOLLIDAY: Unless you assume that they pull chloride from some other site.

MEMBER: You can expand volume without chloride content. We showed that last year.

DR. HOLLIDAY: All right, but you would expect some decrease in concentration, and we know the concentration at the time we pull the chloride out; we know the concentration at the end of the experiment; actually it goes up a little, which means they have contracted rather than expanded.

MEMBER: Your chloride concentration is higher and your bicarbonate is up?

DR. HOLLIDAY: Well, you see, when you put the sodium bicarbonate into the peritoneal cavity, as you take it out you have a measure of serum chloride at that point, assuming it is in equilibrium, and there is ample data to make that assumption -- and it averages about 75. You withdraw by that technique 20% of body chloride. At the end of the experiment, the chloride concentration is in the neighborhood of 80 to 85, so that certainly it doesn't go down; if anything the direction is up.

MEMBER: What is the chloride concentration in these rats which you start with? Did you say 75 meq./day?

DR HOLLIDAY: That is at the end of the peritoneal dialysis. I assume you are familiar with this technique, maybe I should review it for you.

You take a normal rat, inject into him 10% of his body weight of an isotonic solution of sodium bicarbonate, and leave it there for 2 hours. At the end of that 2 hours, the chloride has exchanged for bicarbonate until equilibrium is reached, and we now, so to speak "trap" 20% of his body chloride in the peritoneal cavity. It is at that point that the concentration throughout the extracellular fluid is 75. We now remove

this peritoneal fluid and have a net deficit of chloride, and the concentration from that point on, if anything, tends to rise; that is why I say that the edema is an unlikely component at this stage. But I think it is not because they do not want to develop edema, if you will excuse the teleological aspect of it -- but rather they haven't got anything to develop edema with.

Now I do not know whether the renal lesion is due to the alkalosis, and whether it develops in the state of dietary potassium deficiency as a result of the alkalosis, or whether it is related to the exchange of sodium and potassium within the renal cells. We have experiments designed to try to test these phenomena, and I think any remark now would tend to be more speculative than anything else.

The second aspect that has occurred to me was in reference to the rats on the electrolyte-free diet which we used as our controls. When we observed this drop at 3 days of diet, without any elevation in bicarbonate of serum or of muscle sodium, we then extended the studies. We had rats sacrificed at 3, 6, 12, 28, and just yesterday, 91 days. I can report on all but the last group, they did not have any elevation of bicarbonate over that entire period of time. Their muscle potassium after the first 3 days dropped very slowly, but did continue to drop. The average figure for the 28-day group was I think 39 millimols per 100 gms. of dry fat-free tissue The muscle sodium showed a very slight tendency to rise It was not anywhere in the order of magnitude that you might expect in the classical potassium deficient rat; neither did they have any renal lesions

MEMBER· How about the chloride?

DR HOLLIDAY In those rats it was normal.

MEMBER· What were they drinking?

DR HOLLIDAY Distilled water

MEMBER· They didn't get a low sodium intake?

DR HOLLIDAY· No, those rats didn't. Now I think, Bob, you have found that when feeding rats sodium chloride they did not develop potassium deficiency, Isn't that right?

DR COOKE You get potassium deficiency but you don't get much alkalosis

MEMBER Did you get a marked reduction in muscle potassium and an increase in muscle sodium?

DR COOKE· It depends on how long you go and how fast the rat is growing. I think that makes a big difference

DR HOLLIDAY Yes, but I think probably we ought to eliminate the problem of growth because that, in effect, presupposes a necessary depletion of potassium. We have so far only two groups to report on who were given sodium bicarbonate, as a

load, during the 28-day period on the electrolyte-free diet. They had muscle potassiums down around 27 millimols per hundred grams fat-free solids, which is a rather low figure, extensive alkalosis, and the renal lesions, ---a standard reproduction of the old experiment.

What I think is indicated here is to try different anions and see if these have an effect. We have not noticed any glomerular lesions in the alkalotic rat. Ours is a rather crude technique in view of the histology we have seen here in the last few days, and I say this blushingly, but I do think we may find with our high salt diet, or with some other anions we will add, that this renal lesion has some bearing on the rat who already has nephritis.

I think it would be interesting to try a load of potassium bicarbonate on the rat who is on this electrolyte-free diet, who doesn't get the chlorides, and see if the anion has any relation to potassium secretion, which I think it certainly does. I wouldn't like to postulate exactly what it is

DR METCOFF· What was the result when you gave the potassium and sodium bicarbonate together?

DR. HOLLIDAY. The potassium had no effect. It was just as though we had given them sodium bicarbonate alone; the potassium bicarbonate did not protect the animal against alkalosis, potassium deficiency, and increased muscle sodium, or the development of the renal lesion. No, I am sorry, I gave you a little more data than I should have. They were the rats who were depleted of chloride, and given this load for 3 days. I do not know what would happen if we had not depleted them of chloride It is a question of which comes first, the cart or the horse -- the alkalosis or the potassium deficiency. In the potassium deficient rat, it is still hard to know what leads precisely to the elevation of serum bicarbonate. It is a little easier to go at it the other way; produce alkalosis, and get the potassium deficiency. Certainly everyone has long since been agreed that if you take sodium in some form, you get potassium deficiency and alkalosis on the diet alone.

DR. METCOFF· *Is some hydrogen ion going in as well as sodium to make up the* cation structure of the muscle -- the muscle pH is decreasing at the time this serum pH is going up.

DR HOLLIDAY: I approached that with a very indecisive sort of attitude. The thing that interested me in our rats long-term fed on the electrolyte-free diets was that nothing happened either to the muscle sodium or to their serum bicarbonate, but there was 15% reduction in muscle potassium, or in terms of a molar concentration around 25 milliequivalents. I don't know what happened -- it is possible some organic acid came out with it, I know Dr. Cooke is engaged in studies of that, and I wish I was,s too.

DR COOKE: I wish I wasn't. (laughter)

DR. HOLLIDAY: For reasons beyond my control I am a little removed from it.

DR. COOKE. Could I say a few words about this problem. I think there is a great deal of confusion about why there is alkalosis and why there is acidosis under a given set of conditions. I think that this is made needlessly complex. I am afraid that I am responsible for the concept of hydrogen moving into cells in exchange for sodium and potassium I think in a way that it is unfortunate that it has gotten a great deal of emphasis. We originally suggested this to keep our anions and cations balanced. That is, if we had cations moving out and there seemed to be no change in anionic structure, it was thought that something must have exchanged. Since they were not fixed cations, we assumed that a manufactured cation such as hydrogen ion must have exchanged.

As far as acidosis and alkalosis is concerned, one has to remember that the composition of extracellular fluid in the animal that has a kidney is determined by reabsorption by the kidney. For example, an adult filters about 180 L per day. Out of the 180 liters, 179 L are brought back. This is the equivalent in actual operation of an infusion into the adult of somewhere in the neighborhood of 15 times the extracellular volume. If the material which is being reabsorbed differs in any way from the previous concentrations of electrolyte in the animal then we will very quickly have a marked alteration in extracellular composition These are self evident truths. Essentially, the kidney is perfusing the whole body with its reabsorbate If the reabsorbate is changed in composition, then the whole of extracellular fluid is altered and what happens in exchange between muscle and extracellular fluid is relatively insignificant.

Therefore, I think one must approach the problem of alkalosis and acidosis in any sustained state with the concept that something alters the relationship of sodium to chloride as it is brought back in the tubular reabsorbate Until we recognize this particular phenomenon, we are going to be completely confused by operations of much smaller magnitude that go on between muscle and extracellular fluid and extracellular fluid and the outside world, such as ingestion of a small amount of salt, etc.

What we have been trying to do is get information about states that alter the ratio of sodium to chloride in tubular reabsorbate We have demonstrated, fairly successfully, I think, that in the potassium deficient animal there must be an alteration in the way sodium and chloride are brought back in tubular reabsorbate. If one puts in some potassium, no matter what the anion is, and corrects the potassium deficiency, the ratio of sodium to chloride in reabsorbate is altered and correction of extracellular alkalosis results. If one takes an animal with a sodium concentration of 140 meq./L of serum ultrafiltrate and a chloride concentration of 90 meq./L and potassium bicarbonate is injected without intake of chloride, after five or six days chloride concentration rises to 110 meq /L and sodium remains at 140 meq./L. The extracellular alkalosis is corrected by the administration of potassium bicarbonate.

These experiments cannot be interpreted as a correction on the basis of exchange between cells and extracellular fluid. It has to be explained in terms of some alteration in the way the kidney is handling sodium and chloride We do not pretend to know how this alteration occurs We are working with the idea that the bulk transport of sodium and chloride may not be fixed at constant ratio as assumed by many renal physiologists. Considerable contradictions now exist in regard to the proximal reabsorption of sodium and chloride. Dr. Homer Smith's wonderful book on the kidney indicates that by micro-puncture technique the concentration of chloride rises in the tubular lumen

along the course of the proximal tubule. In addition, this fluid is said to be iso-osmotic. If carbon dioxide is freely diffusible across the proximal tubule -- and I believe most people believe this, and if chloride concentration rises, the fluid cannot be isohydric as stated since bicarbonate concentration would have to be quite low. It is our feeling that some ion such as potassium which alters the intracellular environment of the renal tubule may alter the bulk transport of sodium and chloride in relation to each other.

DR. METCOFF: The significance of these observations is obvious to everyone. They seem particularly important because of the suggestion that a renal lesion, particularly a tubular lesion, associated with edema, may occur in the absence of a specific nephrogenic insult. The original work, as I understood it, was described many years ago by Follis and others who were studying the effect of nutritional deficiency in rats; they also added the observation of extreme fragmentation of the myocardium as one of the characteristic features of potassium depletion.

I wonder if Dr Holliday would agree to the possibility of a storehouse for available chloride. It is conceivable that chloride can be removed from some reservoirs in which it resides at higher than plasma ultrafiltrate concentrations to cause an increased concentration of chloride in extracellular fluid.

DR. HOLLIDAY I want to agree with Dr. Cooke that I don't think sodium and chloride has to be reabsorbed in any fixed proportional manner. I would like to emphasize that this transport is certainly influenced, if not controlled, by cellular composition within the kidney. The best source of measuring cellular composition is muscle, admittedly a crude and sometimes erroneous source. The purpose of bringing to the forefront today the effect of various electrolyte loads, small though they might be in terms of volume of reabsorbate is that it is perfectly evident they very seriously influence this transport system. By using these rather extreme and peculiar loads we hope to bring out the effect of change in sodium and chloride reabsorbate, either after a later change in cellular composition or the reverse phenomenon where cellular composition influences reabsorbate.

Finally, if there is a tie-in here between antigenic renal disease, or antibody-renal disease, and so to speak, electrolyte renal disease, we might be able some day to relate the two.

IV. Therapy

CHAIRMAN RAPOPORT: We are particularly concerned with new forms of therapy of the nephrotic syndrome in children and with an evaluation of the effects of treatment on the course of the disease. Dr. Greenman will start the discussion with observations made in Pittsburgh by himself and Dr. Danowski.

DR. GREENMAN: During the past two years thirty children with the nephrotic syndrome have been treated at Children's Hospital of Pittsburgh. Therapy has been divided into two phases: 1) the delivery of edema through rigid sodium restriction with or without the administration of dextran, polyvinylpyrrolidone or urea; 2) an attempt to alter the course of the disease by the administration of ACTH alone or in combination with nitrogen mustard.

The children ranged in age from 1 year and 11 months to 14 years and 8 months; 20 were males and 10 females Eight were less than 3 years old; 12 were between 3 and 5 years; 8 between 5 and 8 years, and one 11 years 7 months and the last one 14 years 8 months.

The patients were ill with the nephrotic syndrome for 18 days to 22 months prior to hospitalization. Six had had the disorder more than one year; another six between 6 months and 1 year; two between three and six months; and the majority (16) less than three months

Twenty-two of the children had a history of an upper respiratory infection within one month of onset of their illness Only one patient had B hemolytic streptococci in his nose and throat cultures. Antistreptolysin O titers were 12 units per ml in the nine patients in whom they were measured

Blood pressures were within the normal ranges for age in seven patients; in 21 they were below 120 systolic but slightly above normal values for age, and in two others the readings were increased to 130/90 Blood pressure records were difficult to evaluate because of their variability It was our impression that only the latter two children had definite hypertension No patient had cardiac enlargement nor abnormal eyegrounds; the livers were palpable in all Twenty-eight patients had gross edema when admitted, the other two gave definite histories of edema and like all of the others had unequivocal urinary and biochemical abnormalities

All patients had profuse albuminuria (4+) as measured by acidification and heating. None had gross hematuria nor fixed specific gravities Microscopic examinations were carried out after centrifuging a portion of the first voided specimen in the morning at 500 revolutions per minute for 5 minutes. Irrespective of whether red blood cells were or were not seen a benzidine test was performed on the spun sediment. Only two patients were entirely free of both casts and red blood cells All the rest had either one or both abnormalities.

Blood non-protein nitrogen values were elevated above 39 mgm.% in 7 patients; 40 mgm.% in 2; 48 in one; between 52 and 59 in three, and 70 in one other. In the other 23 patients the NPN was within normal limits.

Serum proteins were analyzed by the macro-Kjeldahl method using the Majoor modification for separation of albumin and globulins. Of the 29 patients who had serum albumin values less than 2.0 gm.%, 23 were less than 1 gm.%. One patient had a value of 3.5 gm.%; he was one of the two without edema and his serum cholesterol was 327 mgm.%. He did have however a 4 plus albuminuria and microscopic hematuria and casts. His blood pressure readings were completely normal.

Serum cholesterol values (Lieberman-Burchard method) were greater than 500 mgm.% in 26 patients; in 2 they were above 400 mgm.%; in one 327 and the final patient 264 mgm.%.

The therapeutic regimen was as follows: All patients were placed on diets rigidly limited in sodium to 2.2 to 9.0 meq. per day. The daily intake included about 150 meq. of potassium in the diet, 3.0 grams of protein per kilogram body weight and adequate calories. Supplementary iron and vitamins were given.

The patients were kept at bed rest and antibiotics were given prophylactically, usually penicillin orally or intramuscularly The oral dose was 100,000 units three times a day; the intramuscular one 400,000 units per day. A few patients received either terramycin or aureomycin; one child was treated with streptomycin for a short interval when she developed peritonitis.

Several diuretic agents were used prior to therapy. Two patients ingested urea prior to ACTH therapy. Eighteen patients received colloid either as dextran or polyvinylpyrrolidone in amounts varying from 250 ml. to 7200 ml. Usually the patients were given 500 ml. a day during a 4 to 6 hour interval Preceding and during the administration of the fluid, the blood pressure, the pulse and the respiratory rates were recorded at 15 minute intervals The patients remained under continuous observation. Temperatures were recorded at half hour intervals The body weight, urine volume, relative blood cell volume, hemoglobin level in addition to blood and serum constituents were measured prior to and immediately after infusion as well as 3 and 24 hours later. Chest x-rays for heart size were obtained before, immediately following as well as on the morning after infusion.

ACTH (Armour or National Drug) was given intramuscularly to all patients in a dosage of 25 mgm. every 6 hours for 28 days. The last five patients have received ACTH alone; the other twenty-five were given nitrogen mustard in addition. The dose of nitrogen mustard was 0.3 mgm. per kilogram body weight. This was injected into the tubing during an intravenous infusion of 500 ml of 5% glucose in water. The patients received nothing by mouth 4 to 5 hours before as well as after the injection and were frequently given a sedative dose of phenobarbital beforehand. Blood counts were determined on the day before, the day of, and the day after injection. Usually the nitrogen mustard was given on the third day of ACTH therapy; though in a few instances it was given at the end.

The following observations were recorded on each patient in addition to a daily physical examination with particular attention to the patient's mental and emotional status. Daily blood pressures were taken by nurses usually before the 9 a.m. dose of ACTH with frequent checking by the physician. At times wide fluctuations were found

between these readings. Daily body weights and admission and final heights were recorded. Rectal temperatures were taken three to four times a day. At weekly intervals and at times more frequently the patients had nose and throat cultures, complete blood counts, Wintrobe sedimentation rates, and blood drawn for analysis of blood non-protein nitrogen and sugar, and serum total carbon dioxide content, chloride, sodium, potassium, total protein, albumin, globulins and cholesterol levels. Blood eosinophils were counted On admission tuberculin skin tests, serological tests for syphilis and in one-third of the patients antistreptolysin O titers were determined. Erect teleoroentgenograms for heart size and shape in addition to electrocardiograms were obtained prior to therapy Some time before discharge the kidneys and ureters were visualized by intravenous pyelography. Daily morning specimens of urine were analyzed for specific gravity, acidity, albumin, sugar and microscopic findings. At intervals urine was collected for 24 hours and analyzed for protein and sodium.

Twenty-eight patients were grossly edematous on admission In nineteen of them attempts were made to deliver the extra fluid by use of dextran, polyvinylpyrollidone, urea and stringent sodium restriction In 15 patients (79%) such therapy was successful and in 4 others it failed Twelve (75%) of 16 edematous patients receiving colloid (15 dextran and 1 polyvinylpyrrolidone) delivered their edema during this treatment without adverse effect. The two additional patients, who were not edematous but received dextran, had no decrease in body weight One of the twelve received dextran again 7 months later with complete delivery of edema increasing the success rate to 76% The decrease in body weight frequently took several days to appear; although facial swelling and peripheral pitting decreased early We attributed this to movement of fluid into the circulation from the interstitial spaces in response to the colloid. Three patients delivered their edema prior to hormonal therapy while on diets rigidly restricted in sodium; although one of them required the addition of urea. One other child who had not responded to dextran received urea with only a gradual increase in urine output and a slight fall in body weight He was still edematous at the time ACTH was started The above three patients, who did not receive dextran, had minor decreases in serum sodium concentrations as they became edema free; sodium concentrations were 132, 126 and 133 meq. per liter respectively. No adverse effects were observed. These values returned to normal promptly after starting ACTH while continuing the low sodium diet.

Five patients required paracenteses for relief of discomfort at some time during treatment; *three of these were performed during ACTH therapy*

No attempt to deliver edema, other than to limit sodium, was carried out in the 8 other patients

Fourteen of the thirty patients were edematous at the onset of ACTH therapy. Four had sufficient ascites to require paracentesis prior to the administration of hormone and three (two of the above 4 plus one other patient) accumulated edema while receiving ACTH so that paracenteses were required in the midst of treatment. There was thus a total of five patients on whom this procedure was performed. It was our impression that the paracenteses were hazardous if the patients then reaccumulated edema during periods of strict sodium restriction since serum sodium values would fall alarmingly as the ascites reformed

Five patients received ACTH without nitrogen mustard while 25 received nitrogen mustard as well. Almost all the latter developed nausea and vomiting during and for a period of hours after the nitrogen mustard. None developed bone marrow depression or leukopenia. Subsequently four of the group receiving combined therapy were re-treated for a recurrence or an incomplete response. One patient during re-treatment received ACTH alone and the other three were again given combined therapy.

At the end of the initial course of treatment, 15 patients had completely normal urinalyses and 7 others had 2 plus or more albuminuria. Of these 7, 4 had a few granular casts, 2 had microscopic hematuria and one a normal microscopic examination. In the remaining eight a trace or a 1+ albuminuria was present in all with granular casts in two and microscopic hematuria in 2 others. Serum albumin values were 3.5 gms.% or more in 12 patients, between 3.0 and 3.5 gms.% in another 8, between 2.0 and 3.0 gms.% in 5, between 1.0 and 2.0 in 2 and less than 1.0 gms.% in one. In the remaining 2 of the 30, data on this point are not available. Serum cholesterol was less than 301 mgm.% in 13 of 28 patients in whom measurements were available, 301 to 351 in another 5, 351 to 400 in 4, and between 400 and 600 in 6.

In summary only six patients had normal urines and normal concentrations of blood and serum constituents at the end of therapy; three others had normal urinalyses and serum albumin levels but cholesterol values were elevated between 417 and 457 mgm %; three others had normal urinalyses and serum cholesterol values but low serum albumin levels, 2.7, 3 1 and 3.4 gms.%; the other three patients with normal urinalysis had both albumin and cholesterol abnormalities. Two patients ended the course of therapy with distinct hypertension; in one it subsided after a short interval and seemed definitely related to the ACTH

The patients were followed in our outpatient department at first at two week and then one month intervals for the first three months and then at 3 month intervals. Total duration of follow-up now extends from 1 to 24 months.

Two of the children who did not recover were re-treated several months and one year after the first course Both ended their second course with one plus and two plus albuminuria, normal microscopic examinations and slightly elevated serum cholesterol concentration In one the serum albumin was normal.

Two other patients were re-treated for definite recurrences after remaining normal for a period of three to seven months. The one treated after seven months is again completely well with a follow-up of only three months. The other patient has now undergone his sixth course of therapy with complete clearing of abnormalities one month after his last course. At one time he received cortisone to prevent relapse but the amount, 50 mgm. a day, proved inadequate. The recurrences in this patient appeared at three to six month intervals following upper respiratory infections.

At the last examination of this group of patients 18 (60%) of the 30 were normal upon examination, as well as by virtue of negative urinalyses and biochemical findings; 8 (27%) are greatly improved since 6 have only single abnormalities; traces of albuminuria in two, one plus albuminuria in three, a cholesterol of 361 mgm.% in another; the other 2 have no known abnormalities at present but one of them has not been seen

following his 2 month clinic visit and a letter from his mother 4 months after treatment suggests that the urine is normal. The other patient has just recovered from his sixth flare-up and has been free of albuminuria with normal biochemical data for one month. There are only 4 (13%) children who are definitely abnormal. Two have hypertension and abnormal urines as well as abnormalities of serum proteins and cholesterol; one has a slight evelation of his non-protein nitrogen. One was last seen one month after discharge from the hospital and the last patient still has a nephrotic syndrome but is non-edematous and shows continual slow improvement in biochemical abnormalities.

Only ten patients have been followed one to 2 years after completion of ACTH. Of these 7 are normal; 2 are greatly improved with one plus albuminuria as their only abnormality; and the tenth child has just been re-treated for the sixth time with complete disappearance of all signs of illness again during the ensuing one month.

We have not established any benefit from the addition of nitrogen mustard to our therapy since there have not been any relapses in the six patients treated without it. These six were the last patients treated and are apparently well.

Of the fourteen patients who had edema when ACTH was started, 11 lost the edema with one to two weeks of hormone therapy; one gradually delivered his fluid so that he was non-edematous at the end of four weeks, two others had a diuresis after ACTH was discontinued. Three (including the latter two) required paracenteses during treatment.

The principal complications of therapy were sodium depletion and central nervous system manifestations Despite the rigid sodium restrictions only three patients of the entire group of thirty developed serum sodium concentrations below 132 meq per liter during or after ACTH therapy. One had been edema free prior to administration of ACTH; the other two were definitely edematous. Serum sodium fell to 124 meq. per liter in one child after 2 days of ACTH. He had had a paracentesis 4 days beforehand and was reaccumulating edema and ascites despite a diet containing 2 5 meq. of sodium per day The ACTH was discontinued, he received 500 ml. of 6% dextran in normal saline with a diuresis and increase in serum sodium to 132 meq. per liter. He was re-started on ACTH plus sodium restriction 10 days later and had no subsequent decrease in serum sodium and gradually lost his edema.

The other 2 patients had convulsions One had been treated with ACTH several months earlier with the occurrence of a serum sodium of 120 meq. per liter after 8 days of ACTH In addition he had a severe headache with definite hypertension so that therapy was discontinued He was readmitted because of persistent abnormalities. During the second course of ACTH he gained approximately 1 kilogram body weight per week despite a diet rigidly limited in sodium Edema had not been recognized on admission. On the sixtieth day, following an injection of nitrogen mustard, he developed severe convulsions which produced anoxia Unfortunately he developed cerebral atrophy. His serum sodium was reduced to 112 meq per liter at the time and he had hypochloremia and alkalosis with a low serum potassium concentration. Five days earlier his serum electrolytes had been normal and his urine contained less than 5 meq. of sodium per day He had marked hypertension during this interval Thirteen months later he has no evidence of kidney disease.

The third patient had been ill for 13 months before therapy and had been resistant to diuresis with colloid. He had been troubled with diarrhea. Unfortunately no urine collections were carried out. He retained fluid however even though his diet was rigidly limited in sodium. After 9 days of ACTH his serum sodium was 116 meq. per liter and his blood pressure was greatly increased. He had a severe convulsion that day which disappeared with prompt treatment with phenobarbital, desoxycorticosterone acetate and hypertonic saline. ACTH was re-started 3 weeks later and in 3 more days he had another convulsion with hypertension and serum sodium reduced to 125 meq. per liter. He has had no further ACTH. His Intelligence Quotient subsequently has been better than normal, but he is apparently developing chronic glomerulonephritis.

We were impressed with the number of patients who maintained completely normal serum sodium values. In addition those subjects with abnormally low sodium concentrations prior to ACTH therapy developed normal levels as treatment continued despite the very low sodium intake The patients who developed hyponatremia were the very few who retained fluid despite sodium restriction. Our normal ranges for serum sodium varied from 136 to 151 meq. per liter with a mean of 144 in a group of 59 normal children in the same age range.

Other complications of therapy were mental depression in one patient requiring cessation of treatment after two weeks This patient is well now after re-treatment. Peritonitis developed in one child after one day of ACTH. It responded to streptomycin, penicillin and cessation of ACTH. Subsequently ACTH was re-started without difficulty and the patient responded well. Upper respiratory infections were our most common problem but antibiotics were given and treatment was continued. Many patients had minor increases in blood pressure but in only two was it extreme. One patient developed temporary carbohydrate intolerance which did not require insulin and disappeared shortly after therapy was discontinued. All the children developed moon facies, protuberant abdomens, and cervical fat pads. Only 3 patients developed striae; the striae faded subsequently but did not disappear. Acne appeared in the older children and in a number of patients hirsutism was temporarily evident.

In almost all of the patients body weights temporarily decreased during the first week of therapy; it then gradually rose without the accumulation of edema. Appetites became voracious in those who did well; they wanted to eat continuously in contrast to those who did not recover.

In summary, within the limit of the relatively short duration of our follow-up period 18 (60%) of 30 patients admitted with the nephrotic syndrome are entirely normal; another 8 (27%) are greatly improved and are probably normal and only 4 (13%) are definitely abnormal. Two of the last 4 have not been adequately followed. This seems a better record than previously achieved but a much longer follow-up time is needed before conclusions may be drawn about incidence of recovery. There is little doubt that all patients are symptomatically improved and that ACTH therapy does more than deliver edema Treatment seems safe when the patients are adequately followed in the hospital. Accumulation of edema when patients are receiving ACTH and are on a very low sodium intake seems to be a danger signal.

CHAIRMAN RAPOPORT: How many days of dextran did it take to deliver the edema?

DR. GREENMAN The time varied from one to eight days The end point was judged by both clinical and weight changes. The patients lost up to 25% of their body weight.

CHAIRMAN RAPOPORT: You discontinued it on some of the patients?

DR GREENMAN: We did when we could not demonstrate further beneficial effect.

DR LANGE How quickly did they reaccumulate edema? And how many of them reaccumulated edema within a short period?

DR GREENMAN We usually did not wait for reaccumulation of edema but went on to ACTH therapy at the end of the dextran phase Two patients did regain fluid when a week elapsed between the two phases. I would expect the effect to be temporary since dextran is lost in large amounts in the urine.

MEMBER· Were there other reductions in serum constituents with dextran?

DR. GREENMAN Yes, hematocritis, hemoglobins, serum proteins and cholesterol decreased as a result of plasma expansion. These indices returned to their initial values within several days, however.

MEMBER May I ask were these qualitative tests for urine protein? Or, were they quantitative?

DR GREENMAN: They were both, but mainly qualitative.

MEMBER When did you give nitrogen mustard?

DR. GREENMAN: Usually on the third day of ACTH treatment but in a few instances at the end

MEMBER· Did the heat bother these children, much?

DR GREENMAN· No

MEMBER. This was a 50 mg. sodium diet?

DR GREENMAN Yes

MEMBER Independent of their size?

DR. GREENMAN Yes.

MEMBER· That's a rice diet?

DR. GREENMAN: No, it is much more. It contains fresh fruits, Lonalac, many vegetables, certain cereals, sugar, salt-free bread, jelly, salt-free butter and at times desserts, peanuts and electrolyte-free protein supplements, et cetera.

MEMBER: Do they drink Lonalac, your patients?

DR. GREENMAN: Yes. (Laughter)

MEMBER: Had any of these children received ACTH before?

DR. GREENMAN: Yes, three of them at other hospitals with transient benefit.

MEMBER: Did these patients on a low salt diet who had no edema before the start of ACTH, gain weight during ACTH?

DR. GREENMAN: Yes, but not at first. They usually lost weight in the first 4-5 days. Their dietary intakes became so large, however, that we attributed their gain to an increase in flesh.

MEMBER: What was the chloride content of the diet?

DR. GREENMAN: I don't know exactly, although it must have been high because of the large amount of potassium ingested.

MEMBER: Was the potassium given as potassium?

DR. GREENMAN: Rarely, since the diet contained 150 or more meq. of potassium per day.

MEMBER: Did you find that the restriction of sodium intake was an essential part of your therapy?

DR. GREENMAN: We thought it was important

CHAIRMAN RAPOPORT: In actual practice, on the outside, you are apt to run into such great difficulties in limiting salt that we have practically abandoned doing anything about it, and are willing to settle for the initial rise in weight in the first 5 or 6 days that comes from a relatively free salt intake, or the just slightly reduced one, knowing that it will be delivered along with the rest of the edema. But the ultimate result doesn't make any difference. I think you can remove a lot of the burden of management of patients if you agree that all relatively free salt intake does is increase the sodium retention, but it doesn't make any difference as far as the disease is concerned

DR. GREENMAN: I'm not so sure that it isn't essential to restrict sodium stringently in these children if you are to give the moderately large amounts of ACTH they received. I would have to see data proving the safety and equal benefit of a larger intake of sodium under these circumstances before agreeing with you, especially since there is much basic evidence indicating the importance of sodium restriction

to patients receiving large amounts of this hormone.

MEMBER: Were all of your patients treated in the hospital?

DR. GREENMAN: Yes

DR. METCOFF: Were you planning to present some of the balance data which you talked about, if not, I wonder if the accumulation of edema was proportional to the amount of sodium which was retained?

DR. GREENMAN: No. The patients who accumulated edema were on essentially sodium-free diets and could not have retained sodium.

MEMBER: Did you study steroid output at all?

DR. GREENMAN: We have some data on urinary 17-Ketosteroid output. 17 Ketosteroids increased greatly during therapy and then returned to low levels at its termination.

MEMBER: Were the patients without nitrogen mustard just as depressed mentally as the others.

DR. GREENMAN: Yes. Depression was difficult to evaluate because of homesickness, dietary restrictions, and multiple injections each day.

DR. McCRORY: Would you mind repeating your follow-up data?

DR. GREENMAN: Ten patients have been followed one to two years after cessation of therapy, 11 between five and nine months, and 9 between one and three months.

MEMBER: When you say they have been followed from 12 to 24 months, does that mean they were normal for that length of time?

DR. GREENMAN: No, since several continued to show improvement after discharge from the hospital. Six have been normal for 12 to 18 months, 7 between 7 and 11 months; 2 between 2 and 4 months, and 3 for one month. Those who were definitely normal had no albuminuria

MEMBER: Did the two children that developed convulsions show evidence of a longer standing fixed renal impairment?

DR. GREENMAN: They had been sick 12 and 13 months prior to therapy. One had hypertension. Neither had a fixed urinary specific gravity; one had a blood NPN of 45 mgm.%; the other one had a normal NPN.

DR. McCRORY: Did you have any problem with interfering infections during the 28 days?

DR. GREENMAN: Yes, but it was not serious.

DR. McCRORY: Then you had no infection or elevation in temperature?

DR. GREENMAN: There were a few patients with minor increases in temperature.

DR. McCRORY: You treated intercurrent infection without stopping ACTH?

DR. GREENMAN: Yes, but we added additional antibiotics to our prophylactic one. We use oralppenicillin, 100,000 units three times a day or intramuscular *penicillin*, 400 000 units a day.

DR. McCRORY: What is the ward unit that you treated these patients in?

DR. GREENMAN: There are several. They may be on our own metabolic ward which is set up for special studies or on the general medical floors.

DR. BARNETT· How long has your clinic been following patients with nephrosis?

DR. GREENMAN: Two years.

DR. BARNETT· You said that 6 of them had had the disease for more than one year. Do you remember how long they had had it?

DR. GREENMAN: Two were ill for 12 months; two 13; one 16, and one 22 months.

DR. BARNETT You see, I think many of us who have been following groups like this with treatment for maybe three or four years now are sort of left with a large group of children who have had treatment and who still have the disease, where the duration of the disease, often with reduced kidney function, etc. is 2, or 3, or 4, or 5 years. Dr. McCrory just pointed out that the first patients we treated, who seemed to have done better than the ones we have been treating the last year or so, did get more *hormone than we have been giving the last few years.*

DR. McCRORY: I agree with you, we are apparently getting a different kind of a patient. They seem to respond differently to the same therapy, the same dose.

MEMBER: Did these relapses all occur after upper respiratory infections?

DR. GREENMAN: Yes, in both patients. Thus far these are the only relapses we have seen. Do you believe that we may not have a representative group of patients?

DR. RILEY· No, only that you have four children now who are active even though treated -- that is, out of thirty. Now, I think in four years you will have these 4, plus 4 more. The group of patients that we're seeing now, having done this for three or four or five years, are an accumulation of these children that have not responded, rather than an *unselected group of patients. This is my only point.*

DR. GREENMAN Was your therapy the same as ours?

DR. METCOFF: I thought I might mention -- as Henry has suggested -- that it

is very important to see what your patients who are in remission without any sign of abnormality are like about two years after the onset of the complete remission. Those of us who have been following such patients have found to our chagrin that we are seeing rather a large number of such children who, we felt were quite well, now appearing, once again, with recurrent proteinuria and other manifestations. This was unique in everyone's experience, prior to this time. So that it would appear that a very long period of freedom of manifestations of the disease must be present before one should commit one's self as to complete normality.

MEMBER. What is the longest period, Dr. Metcoff, that you have seen a case relapse after being completely free of signs and symptoms?

DR. METCOFF 18 months to 2 years I might also say, Dr. Greenman, that I think your approach has been a unique and a most interesting and very important one for us. I would like to ask one other question pertaining to antibiotics; do you ever see generalized systemic infections due to gram-negative organisms in nephrotics?

DR GREENMAN We had no known gram negative infections.

MEMBER What did your nose and throat cultures reveal? Did you get organisms that were penicillin-resistant?

DR. GREENMAN: Not that we recognized. Several times we cultured organisms from nose and throat cultures which are usually considered pathogens but we were never certain of their significance if there were no systemic or local manifestations. The findings of an organism such as a B. hemolytic streptococcus on routine nose and throat cultures does not necessarily mean that the host is suffering from such an infection We did sensitivity tests only when indicated.

MEMBER Would you like to say a little more about the dextran? You talked about different strengths, which one do you think is the most suitable, now?

DR GREENMAN: It is my impression that a high concentration of colloid without sodium might be the most effective.

MEMBER The 20%?

DR. GREENMAN: Yes, if it were available.

DR DANOWSKI There are others: 10, 12, 20% solutions in water, in 5% glucose, or in saline are available. I should like to speak on the point of sodium restriction. When sodium intake is restricted markedly in rats, hypertrophy of the adrenal cortex develops Hence, if you are going to stimulate the adrenal cortex with ACTH it may be that 100 mgm. of ACTH will do a lot more than 100 mgm. of ACTH on an ordinary sodium diet

The second point I would like to make is that there is no question but that a large group of vascular diseases that are aggravated by sodium are benefited by marked sodium restriction I don't know how to identify them, but I know they exist. There

may. therefore, be something in this marked sodium restriction which transcends the control of edema.

MEMBER: Do you think there is any danger if you use cortisone for a long period of time, that you will get in trouble with adrenal atrophy?

DR. DANOWSKI: We have tried various ways of getting away from it such as intensive treatment for six weeks with 300 mgm. of cortisone a day, and then gradually reducing the dosage between six and eight weeks. At one phase we introduced one week of ACTH without definite benefit. However, in a large series of patients, somewhere around 100, we have not identified adrenal cortical insufficiency.

DR. HOLLIDAY Comments about diet are interesting, but rats, on what is called a low electrolyte diet, deficient in sodium, potassium and also chlorides, will not develop adrenal hypertrophy.

DR. DANOWSKI: Since a low sodium diet produces adrenal hyvertrophy and a low potassium diet results in adrenal atrophy, it may be they cancel one another.

DR HOLLIDAY: A further point, is the anion which goes along with the cation. It has been reported that it is more difficult to get alkalosis with sodium chloride than it is with sodium bicarbonate on potassium deficient diets.

MEMBER: Did you have any patients who had rises of non-protein nitrogen while on therapy and how high did they get?

DR. GREENMAN: Yes, we had some patients who developed transient increases in blood non-protein-nitrogen concentrations during therapy with ACTH. At the same time they had a great intake in food consumption. At no time did the values increase above 45 mgm.% and they would spontaneosly return to normal on continued therapy.

DR SLATER What about the antigenicity of dextran?

DR. GREENMAN: No patient exhibited antigenic reactions. I understand that the drug companies screen the material carefully.

MEMBER: Had they repeated doses of dextran?

DR. GREENMAN: One patient received dextran on a second occasion seven months after his first course; another one had had dextran at another hospital several months earlier. Neither one had reactions.

MEMBER: Whose dextran is that?

DR. GREENMAN: We had Commercial Solvents Corporation, General Aniline, and Laros Company dextran.

MEMBER: Was the volume of dextran infused the same, regardless of concentration? How long was the infusion time?

DR. GREENMAN: Patients received 500 ml. per day of either 6 or 12% dextran. The time taken for the administration of this volume of solution is very important. For example, three children received 500 ml. of 6% dextran in saline within three hours with development of abdominal pain, headache, and hypertension. Subsequently, the fluid was given during 4 to 6 hours without unpleasant symptoms and with only minor increases in blood pressure.

MEMBER: You didn't have any evidence of bleeding tendency, did you?

DR GREENMAN Two youngsters had nose bleeds immediately after administration of the fluid; neither had more than a 10 mm. increase in blood pressure. Both had been picking at their noses so that we were not certain of the etiology of the epistaxis. We had no other bleeding in any patient

DR DANOWSKI Heart size increases following the administration of the dextran in these patients

DR. RILEY: Dr. Caffey has been stressing microcardia for a long time with ours.

DR DANOWSKI: Microcardia could be purely a reflection of course of diminished plasma volume but in this country we do not think of heart size as being related to plasma volume The Swedes and the Norwegians have been smart about it; they have correlated it with pulmonary blood volume and circulating blood volume

CHAIRMAN RAPOPORT: Dr. Kramer will continue the discussion on Therapy by presenting some observations on "two-course" therapy with ACTH.

DR KRAMER: Several years ago Dr Sidney P. Gottfried, Dr John F Steinman, and I(1) published a paper on the chemical composition of the blood of children with lipoid nephrosis during different stages of the disease. Among other things we showed that diureses could occur with no demonstrable change in the chemical composition of the blood plasma. Although there was a critical level of serum albumin or total protein concentration at which edema either appeared or disappeared, nevertheless, with low concentrations of these blood components, striking variation in edema would occur without demonstrable quantitative chemical changes in the blood. These results suggested some other mechanism to be responsible for diuresis at low serum protein levels. Since we were unable to effect a loss of edema at will we had to wait in the hope that spontaneous diuresis would occur in order to correlate the degree of edema with specific changes in the chemical composition of the plasma. With the introduction of ACTH it became possible to bring about diuresis with a fair degree of regularity and thereby to study the changes in the chemical composition of the blood, and to a certain extent of the urine before, during, and after diuresis.

We had several objectives: 1) To confirm previous observations as to the efficacy of ACTH; 2) To determine the optimal dosage; 3) To study the changes in the blood and urine; 4) To see if we could reverse the chemical pattern of the blood, and if possible, even restore that pattern to a completely normal one; 5) Also to *determine*,

(1) Gottfried, S.P., Steinman, J F., and Kramer, B., Amer. J. Dis. Child. 74: 283, 1947

if possible, what chemical changes precede the onset of diuresis so as to foretell when diuresis would occur; 6) To determine the dangers of this method of therapy -- perhaps the first sign of danger --; 7) Eventually to determine both the immediate and the ultimate prognosis as far as the disease itself was concerned; 8) If possible to determine the effect of this form of therapy not only on the renal lesion, but on other organs in fatal cases.

Our studies were limited almost exclusively to observations on the effect of ACTH. At first we selected patients that had marked edema, no fever, hypertension or hematuria. Later, as we learned that some patients even with azotemia and hypertension may improve under treatment, we treated all cases with ACTH and discontinued it in those showing hypertension, a rising non-protein nitrogen or urea in the plasma or with patients who did not otherwise react favorably.

We determined the body weight, the sedimentation rate, non-protein nitrogen of the plasma and carried out frequent determinations of blood pressure, EKG's, as well as measurements of the degree of proteinuria by the roughly quantitative chemical tests. In a few instances we determined the protein partition of the plasma by the method of electrophoresis before, during and after diuresis. We also determined the cholesterol partition and the total lipids of the plasma

Perhaps I will bring out the remaining points by referring to specific illustrative cases.

Fig. 56 indicates one of our early cases. This child suffered from a typical lipoid nephrosis He was gaining weight and had a serum protein level which was not particularly low, a fairly high cholesterol, a 4-plus albumin in the urine. He had been treated with a variety of agents for about 20 days with no effect and then given very small doses of ACTH. The child developed marked discomfort and paracentesis was done. This was followed by a rapid loss of weight, the disappearance of edema, an improvement in the blood composition, and complete recovery. Because of the small dose of ACTH we were skeptical as to the part which ACTH played in this spectacular recovery. On questioning the mother we found that this child had been suffering with Petit Mal for which he had been getting Tridione for a long time. It is possible that this child was suffering from Tridione nephrosis The child has remained well. The Tridione therapy has not been repeated.

Fig. 57 is a rather *typical chart of a child who has responded favorably to ACTH* after a single course of treatment. You see the drop in body weight, the drop in sedimentation rate, the drop in the total and free cholesterol, the decrease in proteinuria, and in blood urea nitrogen, a rise in the plasma albumin, and eventually also in the total protein with a dose of ACTH which varied from about 40 mg. to 100 mg. The sharp drop in weight was followed by a rapid reduction in dosage and discontinuance of therapy Several patients have had similar responses We have had several cases who failed to respond to inadequate doses of ACTH

We thought that perhaps we could prolong the beneficial effect, clinically, and perhaps bring the blood entirely to normal by giving the children a second course of therapy after their diuresis. After waiting a short time to see whether improvement

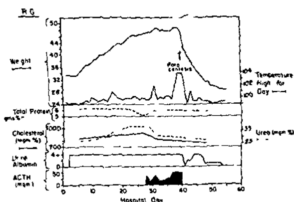


Fig. 56 Response of child with nephrotic syndrome and epilepsy to paracentesis, ACTH and omission of Tridione.

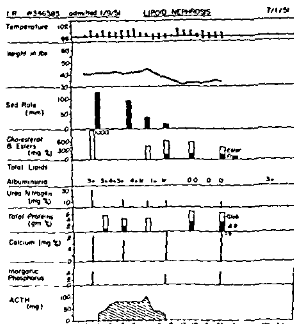


Fig 57. Typical response to ACTH Therapy

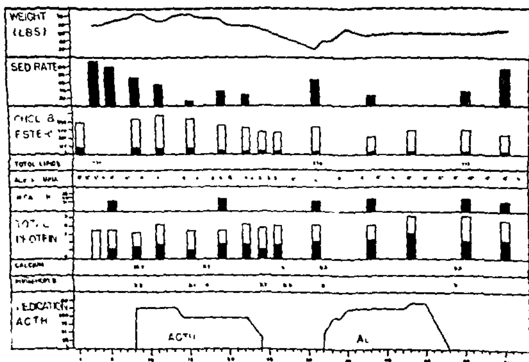


Fig. 58. Dual-course ACTH therapy, typical response

would continue as a result of the first course, we repeated the course of therapy and during this period the children gained weight as shown in a typical instance in Fig. 58. This weight gain was associated with a tremendous improvement in appetite and was apparently due to an actual increase in body tissue and not to edema. The sedimentation rate dropped to a very low level, the cholesterol, cholesterol esters and total lipids returned to normal. The plasma protein level rose to a low normal, and the albumin to about 4.5 gms.%.

We were now able to restore the chemical pattern of the blood with a fair degree of regularity and with discontinuance of therapy to occasionally observe the reappearance of the nephrotic pattern This provided an opportunity to study the mechanism of the hypercholesterolemia and the hyperlipemia as well as that of the hypoproteinemia. We are at present engaged in a study of these phenomena.

We have studied one little boy who had a very severe nephrosis, who was first given the ion absorbing resins, and responded very unfavorably to the treatment. He developed slight hematuria and his edema became worse. He was then given a series of courses of ACTH and responded to the third with a diuresis. He then had a recurrence. Later he contracted measles and responded with a marked diuresis and a disappearance of his edema; after a few months he again developed edema and was given two courses of ACTH with a good response When he went home he still had a 4-plus albuminuria He had a normal urea nitrogen and a moderately elevated cholesterol He has been seen since and is perfectly well, both chemically as well as clinically.

Fig 59 illustrates the clinical course of 8 cases. Note the repeated occurrence of upper respiratory infections followed by the reappearance of clinical edema and the response to ACTH therapy. Previous therapy with ACTH does not seem to alter the ability of the organism to respond to the same dose of ACTH. In most instances hormonal therapy seems to suppress symptoms without affecting the basic pathology

Table 16 is a summary of the chemical findings in the entire group. There may be some question about the serum albumin level because this was done chemically. A number of determinations were done by electrophoresis. This gave much lower values on the same serum. But the general trend is quite obvious, namely, a marked rise in the albumin, which is more marked after the second course than after the first. There is very little if any change, perhaps a slight decrease, in globulin, a rise in the total proteins, a drop in the lipids, a decrease in the total cholesterol concentration, and a rather high free cholesterol, the explanation for which is not obvious.

Table 17 is a summary of the follow-up on 17 of our 19 cases. We had four deaths. Two died of renal failure; one had hypertension; one had base-losing nephritis. Of the 13 living patients that we were able to follow, 6 had no chemical follow-up. We divided them into three groups. 1) the fatal cases; 2) those who were clinically well, but whose blood chemical patterns we were unable to study, and those who were clinically and chemically well. In summary we can say that 21% died; 29% were chemically and clinically well, and about 60% were either chemically and/or clinically well but on whom we had no further chemical data.

Table 16

Average Value of Eight Patients Treated With Two Courses of ACTH

	Pre-Treatment	End of First Course	Beginning of second course	End of second course	1-2 months later**
Serum albumin Gm. %	1.39	2.10	2.61	3.80	4.05
Serum globulin Gm. %	2.69	2.47	2.69	2.27	2.40
Total protein Gm %	4.09	4 87	5.30	6.07	6.45
Total lipid mg. %*	1,770	1,039	---	602	---
Total cholesterol mg %	659	435	357	238	234
Percentage cholesterol estified	36.8	39 6	40 5	35.6	39.6

* Based on determination on four patients.
**Based on determination on six patients. Average value of these six at end of second course was albumin, 3 84; globulin, 2 33; cholesterol, 264, per cent esterified, 39.4.

Table 17

FOLLOW-UP ON 17 CASES OF NEPHROSIS

4 Deaths
- 2 renal failure
- 1 hypertension
- 1 base losing nephritis

13 Living
- 6 NO CHEMICAL FOLLOW-UP
  - 5 clinically well
  - 1 fair
- 5 clinically and chemically well for 11-32 mos.
- 2 poor condition - chemically and clinically

Percentages
- Deaths - 24%
- Chemically well - 29%
- Chemically and/or clinically well - 59%

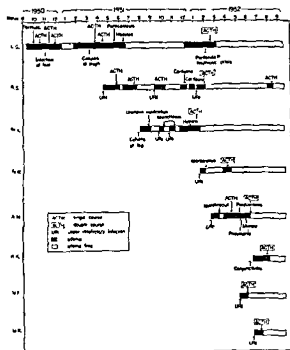


Fig 59: Follow-up of 8 patients treated with double courses of ACTH

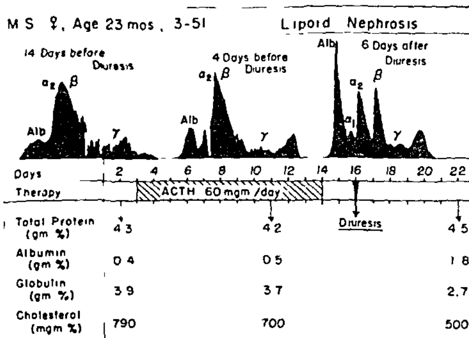


Fig 60: Typical alterations of electrophoretic patterns of plasma proteins before and after ACTH-induced diuresis in the nephrotic syndrome.

Fig. 60 is illustrative of electrophoretic studies we have done. They show the striking improvement in the electrophoretic pattern during and after treatment, but no change in the gamma globulin. With treatment there is a marked increase of the serum albumin peak, combined with a separation of the various globulin fractions and little if any change in the gamma globulin

DR. RAPOPORT: Dr. Lange will you be kind enough to bring us up to date on your studies of complement?

DR. LANGE: I just want to recapitulate quickly a few facts about serum complement levels in nephritis and nephrosis in order to bring those of you who are not familiar with our studies in this field up to date. In addition, I would like to show some recent results from our laboratory which seem to overcome certain objections against our idea that serum complement determinations are an indicator of immunologic activity in nephritis and the nephrotic syndrome. As you may remember, we originally tried to determine antibody titers against human kidney in patients with nephritis and the nephrotic syndrome.

When we studied such cases, we found that in the first month of acute glomerulonephritis, only 58% of the cases showed consistently positive titers against human kidney. During the later phases of the disease, however, 100% of the cases showed such positive titers We assume that in conformity with the animal experiments of Masugi in the early stages of the disease, antibodies are completely absorbed in the kidney when they come in contact with the antigenic surface, namely, the glomerulus or more specifically the basement membrane This would explain well why in the early stages there is an almost complete absorption of the antibody, resulting in low serum complement levels until a certain degree of saturation of the antigenic surfaces has taken place and free

Table 18

DETERMINATIONS OF ANTIBODY TITERS AGAINST HUMAN KIDNEY IN NEPHRITICS AND CONTROLS

	No of Cases	No of Determinations	Positive Determinations Percent	Average Titer of Positive Determinations	Cases Consistently Positive Percent
Total Nephritis	23	122	75	1:623	78
Early Nephritis	12	44	68	1:918	58
Late Nephritis	11	78	78	1:337	100
Controls	68	126	19	1: 78	6

Table 19

PROPERTIES OF COMPLEMENT COMPONENTS

Component	Chemical Characteristics	Solubility	Thermostability	Remarks
C'1	Euglobulin	poor; precipitated by CO_2	-- (32°C)	≈ 0.6% of plasma proteins
C'2	α Globulin	good, CO_2 soluble	- (50°)	
C'3	Phospholipid	good	+	Removed by Heparin, Yeast, E Coli, E Typhi, B prodigiosus
C'4	Carbohydrate with Carbonyl group (coupled with α Globulin of C'2)	good	++	Removed by NH_3, Ether

The objection has been made, especially by Dr. Heymann, to whom I am greatly indebted for his stimulating discussion, that complement may be lost into the urine Although we know that urine of patients with nephritis and the nephrotic syndrome never shows complementary activity, it is very well possible that one of the individual components of complement may be excreted into the urine, thus reducing the complementary activity of the serum, since only the presence of all four known components of complement in sufficient amounts will produce full complementary activity Complement itself consists of at least four components as shown in Table 19. Every one of them has to be present to give an immulogic activity.

Table 19 also shows the properties of the individual components of complement as far as they are known at present C'1 can be separated from C'2 by dialysis against a phosphate buffer C'3 can be removed from the total complex by yeast, while C'4 can be removed by ammoniation One is thus able to produce reagent sera which are devoid of one component of complement only, but for this very same reason do not have any complementary activity. If a test serum or a urine specimen is added to such a series of reagent sera, one can determine exactly the amount of individual complement components which are present in the specimen It is important to know which component of complement is the limiting factor of activity in a given specimen, for we know from the basic work of Pillemer and his associates, (J. Immunol. 47:181-229, 1943), that in an immune precipitate certain components of complement are used up while others are used only to a lesser extent or not at all. Table 20 shows that *in all* cases of acute glomeronephritis or the nephrotic syndrome, it is always component C'2 and to a lesser extent C'4, which are the limiting factors of the complement activity of these sera

which is most severely depressed by a tie-up to the immune complex, all others being depressed to a lighter degree or not at all. C'3 seems to be a catalyst in the reaction. Its presence is indispensible for cell destruction but it is not used up.

As far as our general studies on the effectiveness of certain therapeutic procedures for the relief of the nephrotic syndrome and their relation to serum complement levels were concerned, the following results were obtained· 6-10 days subsequent to the beginning of a course of ACTH treatment (100 mg./day for 7 days to children, 160 mg./day for 7 days to adults), complement rises toward or to normal levels and diuresis starts shortly thereafter The same thing occurs with an identical pattern when cortisone orally (400 mg./day for 7 days) was given to a group of children and adults with the nephrotic syndrome If complement does not rise, diuresis does not occur. Since we have observed that in a high percentage of cases to whom only one course of ACTH or cortisone therapy was given, complement starts to fall again to subnormal levels shortly after diuresis has occurred, with a return of edema, maintenance therapy was used for the last two years in all our cases. We consider the fall of complement levels as an indication of a revival of antibody formation with a concomitant occurrence of edema.

Table 22 shows our results in 14 patients with the nephrotic syndrome who were treated according to the following scheme: Initially a course of 7 days of ACTH or oral cortisone was given Only if complete diuresis occurred, maintenance therapy with either 100 mg /day ACTH for 3 successive days out of each week was started 5 days after the end of the original course, or, more recently, 400 mg./day of oral cortisone were given for 3 successive days of each week, for example, the patients received the maintenance therapy on Monday, Tuesday and Wednesday of each week and were then taken off therapy for the rest of the week and re-started on the next Monday.

We wish to make one basic statement: We have never seen a patient in the 156 maintenance courses so far, relapse with edema, increase his proteinuria or show a fall in plasma proteins while on maintenance therapy. This is so, provided a complete diuresis had immediately preceded the onset of maintenance therapy. In view of certain questions asked, it should be stressed that maintenance therapy seems to be of *value only if and when such a complete diuresis has preceded*. It appears, furthermore, important to stress the significance of giving large doses of steroids, since we know that other groups using our scheme of maintenance therapy, but halving the doses, have seen relapses while on maintenance therapy.

The question of the duration of maintenance therapy is by no means solved at the present time. As Dr. Cooke stated the other day, "I think we have the tiger by the tail but we do not know when to let go". I think that this essentially describes the present situation. We are afraid of letting these patients go out of the maintenance therapy since we have seen recurrences were invariably anteceded by an upper respiratory infection which we feel acts as a booster on the slumbering antibody formation centers. Since maintenance therapy in our experience has been devoid of any major undesirable side effects, we feel at present that maintenance therapy should be continued for many months in order to reduce the chances of a resumption of antibody formation. We have seen, however, symptoms as seen in Cushing's disease after a 7-day course of 400 mg./day of oral cortisone. It should be stressed that each 7-day course of cortisone

in high doses should be terminated by 1 or 2 injections of ACTH in order to stimulate resumption of cortical activity *With this type of therapy we have achieved the following* results: In our group of 14 cases we have had no deaths. One case had edema at the time when this tabulation was made, but the edema occurred many months after the end of maintenance therapy which had lasted for 6 weeks only. The remaining 13 cases had no edema, 3 had no abnormal amounts of protein in the urine, 4 had a trace of protein, 4 had 1+, 1 had 2+ and 1 had 3+ proteinuria. The average time of observation after the beginning of maintenance therapy was 15 months and the average total observation of the cases was 21.6 months We feel, therefore, that maintenance therapy should be given a further thorough study since it seems to offer a better chance for recovery and survival of these cases

CHAIRMAN RAPOPORT: Can we keep the discussion 'til the end? Having said that, Dr. Metcoff, would you like to proceed?

DR. METCOFF I should like to comment about three phases of therapy. The first of these will be the question of sodium restriction; the second will be the use of dextran, and the third, results of ACTH and cortisone, given as single courses.

Table 22

Results of Maintenance Therapy
with ACTH or CORTISONE
after initial diuresis

Case No	Name	At Present Edema	At Present No Edema	Proteinuria	Died	Main Courses	Maintenance Courses	Time of Observation Total	Time of Observation After Beginning of Maintenance
1	H A		X	tr		3	27	20	16
2	R Y.*		X	1+		2	17	31	11
3	T I		X	-		2	20	16	13
4	J K*		X	tr		1	6	11	8
5	A P		X	tr		1	4	32	30
6	R L		X	1+		2	9	28	24
7	E L*	X		4+		2	9	16	13
8	G Pe*		X	1+		1	11	15	13
9	G Po		X	tr		1	6	18	17
10	F R		X	1+		1	5	60	27
11	M R		X	-		1	10	5	4
12	J S*		X	3+		2	11	36	20
13	J T		X	-		2	22	9	7
14	D S*		X	2+		1	5	8	7
No of Cases 14		1	13	neg 3 tr. 4 1+ 4 2+ 1 3+ 1 4+ 1	0	22	156	Average 21.6 mos	Average 15 0 mos

As far as sodium restriction is concerned, I think this is of some importance in relation to the comments made yesterday by Dr. Greenman; I believe that sodium restriction usually is undesirable; I believe that they were fortunate because they had previously diuresed their patients, and employed sodium restriction diets during the time that the children were non-edematous. Had they not done so and put them on severe sodium restriction during the phase of massive edema, I suspect they would have had more instances of severe distortion in body composition.

The role of sodium in edema is often determined on the basis of balance responses. One of the questions which arises in interpretation of balance measurements is that of the skin losses I thought perhaps you would be interested in seeing the results of a study in which skin losses were directly measured. These measurements were made by laboriously washing the skin of the child who was kept in carefully distilled-water-washed clothing, and distilled-water-washed bedding and then concentrating and analyzing the washings from skin and bed clothes. The data were obtained by Dr. Gordillo with the assistance of Mrs. Gadway, our metabolism nurse. The 2 8/12 year old nephrotic child was maintained on balance for some 63 days at fairly constant room temperature. In Fig 62 the sodium intake per day is indicated during several periods on the abscissa. On the ordinate in the top panel are indicated the average daily skin losses in millimols of sodium, potassium, chloride and nitrogen for the period. The skin losses are in the neighborhood of 1 to 1.5 millimols of sodium per day on the average; potassium and chloride are about the same; nitrogen is somewhat larger. You will note that although there appears to be an increased loss of sodium -- approximately 2 millimols per day, during a very high sodium intake, (102 millimols per day) the loss of sodium through the skin does not significantly influence balance measurements during periods of moderate to high sodium intake.

In contrast to this, during periods of extremely low salt intake where the sodium intake, as sodium chloride in this instance, varied from 5 to 6 millimols per day, the skin losses still averaged in the neighborhood of 1 millimol per day. Therefore 1/5th or 20% of the balance was represented by loss of electrolyte through the skin. On a very low salt intake and particularly in periods where sodium excretion is small, skin loss can be a very significant feature of balance measurements.

DR FOX: The child was quite edematous?

DR METCOFF: Yes, he weighed 12.82 kg. at the start of the study, reached 17 8 kg. by the end of the loading period and diuresed down to 11.6 kg. by the end of the study. We estimated surface area according to changing weight and height, and therefore with the changing edema, we felt that we were dealing with a changing surface which might modify interpretation of the data. This did not seem to be so.

Next dextran· We have used dextran rather extensively to produce diuresis. All of our experience has been with the hyperoncotic preparation, 12% dextran in water, which was kindly provided for us by the Commercial Solvents Corporation. We found its effect to be about like concentrated serum-albumin. It was possible to produce a temporary diuresis quite easily with this material, and we have used it as an adjuvant to therapy. We have been interested in the mechanism of the diuresis induced by dextran. Fig. 63 indicates some features of the mechanism of diuresis. This is in a

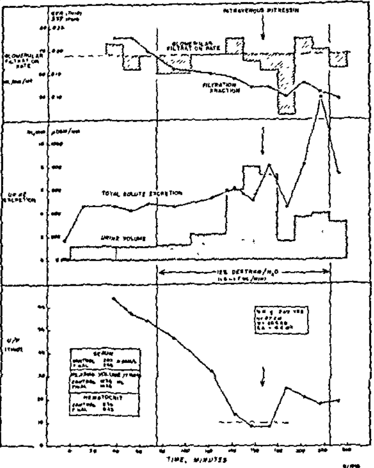


Fig. 62: Average measured daily skin losses of Sodium, potassium, chloride and nitrogen in a child with the nephrotic syndrome.

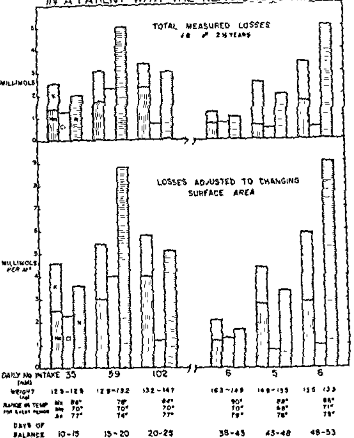


Fig. 63: Effect of pitressin on dextran-induced water diuresis in a child with the nephrotic syndrome.

3 1/2 year old child, height 97 cm., weight 1.6 kg. The glomerular filtration rate was measured with thiosulphate because dextran interferes with the determination of inulin. It is possible to simultaneously determine glucose and fructose, but this is a difficult procedure, and in our hands did not give consistent recoveries. We used thiosulphate at low concentrations and felt that although a slight solute diuresis would be induced, it would not invalidate the results

Along the abscissa is indicated time in minutes. The initial period of control study lasted about 75 minutes During this period of time urine volumes and total solute excretions were measured. U/P ratio for thiosulphate was determined. Glomerular filtration rate is indicated in the top section; this is about normal in this particular child the dotted line refers to the average values of glomerular filtration in the three control periods The filtration fraction is indicated by the line connected by solid dots. During the next periods 250 ml 12% dextran was infused. As you can see, during the first hour or so of infusion, there was no striking change in glomerular filtration rate. Parenthetically it might be added that we have had an opportunity to measure glomerular filtration rate in 4 children during dextran infusion, and in 2 of these it rose prior to the onset of diuresis, and in 2 it did not rise This happened to be one of the children in which only a very insignificant rise was observed The filtration fraction fell progressively There was a tremendous increase in urine volume going from about 0.2 ml. per minute to about 9 ml per minute during the time in which dextran was being infused. This increase in urine volume was not associated with a significant increase in urine solute excretion During this period of time there was a progressive fall in the U/P ratio for thio. The fall in this ratio indicates that a decreasing amount of water is reabsorbed after the water has been filtered This dotted line set at 10 is considered to be the level at which maximum water diuresis occurs It is apparent, therefore, that the increase in urine volume without significant change in the glomerular filtration rate, without change in solute excretion and associated with a fall in solute U/P ratio, represents a water diuresis. Dextran produces a water diuresis

The mechanism by which this occurs remains undetermined. At the point indicated on the chart we gave pitressin intravenously With its administration there was a temporary but significant fall, although slightly delayed, in glomerular filtration rate There was a rise in the U/P ratio, and a cessation of diuresis We believe, therefore, that dextran-induced diuresis is due to inhibition of the effect of circulating pitressin on water excretion The pitressin effect diminished in 15 or 20 minutes, and the water diuresis tended to return. You will note that there was a marked rise in solute excretion by the end of the infusion. Water diuresis is initiated by dextran and this usually is followed by increase in solute excretion. Subsequently, the removal of salt and water may continue with deliverance of all edema We usually administered dextran for from 4 to 6 days So far we have had reasonably good luck in removal of edema, but we have oftentimes encountered severe hypertension, which is our principal cause for concern in this type of therapy

MEMBER· How much do you give?

DR METCOFF: We give 300 ml. per square meter of the 12% dextran in water, usually given in a period of an hour and a half It should be pointed out that the dextran is a kind of polyglot molecule that has an average range of molecular weight between

20,000 and 120,000 -- the cut which we have is probably in the neighborhood of 40,000 to 70,000.

DR. FOX: Was this given daily for 3 or 4 days -- one injection?

DR. METCOFF: Yes, one injection daily for from 4 to 6 days.

MEMBER. Jack, this would mean that a 10 kg. child would get about one-half of that, wouldn't it?

DR. METCOFF: Less than a half, about 100 to 150 cc. in a 2-year old child.

CHAIRMAN RAPOPORT: What were their daily intakes of water?

DR. METCOFF: These were constant During this period of time they were not especially hydrated prior to this procedure, nor was water especially restricted. In other words, they were ordinarily getting between 800 and 1100 cc.'s of water for a *child of about -- our average group runs about 3 years of age.*

Finally, I would like to take a moment to present our follow-up studies on ACTH and cortisone therapy. We have had some experience with these drugs in the past few years, and Table 23 refers to the results obtained in patients who were treated with single courses of ACTH and/or cortisone. Table 23 is so composed that all patients who received one, or possibly two, courses of ACTH in separate courses, are included in the first row; those receiving nothing but cortisone, are included in the second row; and patients who at one time received ACTH, and at some other time received cortisone, are included in the third row. The numbers of patients is as indicated in the first column, a total of 92 patients.

Table 23

Nephrotic Syndrome
Clinical Response to ACTH and/or Cortisone Therapy
(Nov. 1949 - April 1953)

Drug	*Patients*	*Courses*			*Diuresis*	
		6 days or less	7 days or more *	Total	Number	Percent
ACTH	67	14	73	84	64	74
Cortisone	6	3	8	11	6	55
ACTH & Cortisone**	19	9	61	70	49	70
Total	92	26	142	168	119	71

*Usual course was of 10 days duration

**ACTH or cortisone were given as separate couses

We have always given ACTH at a dosage level of 150 to 200 mg. per square meter of surface area per day for 10 days. Cortisone is given at dosage levels of 400 to 500 mg. per square meter per day, for 10 days. None of these results included maintenance therapy. We have divided our group arbitrarily into patients who received therapy for 6 days or less, the 6 days occasioned by the fact that it had to be stopped for one or another clinical reason, such as infection, hypertension, or severe chemical disturbance; or for 7 days or more.

In the latter category, which is the group we think had had adequate therapy, there were a total of 142 courses, 142 of 168 starts. Diuresis occurred in 74% of the patients receiving ACTH, 55% of those receiving cortisone, and obviously, 55% of such a small group has no significance in this regard. Similarly 70% of the last group was also too small a number to have significance. In the overall group, about 71% of the patients receiving ACTH or cortisone therapy appeared to diurese.

Table 24

Nephrotic Syndrome
Status of Patients Given at Least One Course of ACTH and/or Cortisone
During Period Nov 1949 - April 1953

Drug	Patients	Remission*			Active	Dead	Unknown
		Proteinuria Persists	No Proteinuria	Total			
ACTH	67	10	13	23	14	17	13
Cortisone	6	1	0	1	2	2	1
ACTH & Cortisone**	19	4	1	5	7	4	-
Total	92	17	14	31	23	23	14

No edema, clinically well for approximately 1+ years
**As separate courses

Table 24 represents follow-up observations on some of these children. The 92 children have all been followed for a reasonably long period of time. The composition of the chart, as indicated along the ordinate, is the same as the preceding one. "Remission" indicates the presence of no edema in children who have been clinically well for at least one year. We have quite a few additional children in remission for less than a year. Of these 23 children that received only one, or possibly two, separate courses of ACTH, ten of them have some persistent proteinuria but appear to be well; 13 have no proteinuria. Fourteen of this group are active with evidence of the nephrotic syndrome; 17 are dead, and 13 we have been unable to get back for detailed evaluation, and they are considered to be of unknown status. The ACTH and cortisone totals are indicated along the bottom row Of the 92 patients, just about a third appear to be in remission for at least a year following one or two courses of ACTH, and of these about half have

no proteinuria at this time and appear to be clinically and chemically well. These children have had no maintenance therapy.

These data imply that if a large enough series is obtained and followed for a long enough period of time, children may do as well with a single or several single courses of ACTH if they are going to respond, as they do with maintenance therapy. The results do not appear to be as good as those of Drs. Greenman and Danowski.

It has been my experience, just as it has been all of yours, that there are individual patients whom I am perfectly sure have been benefited by maintenance therapy. If there were time, which there is not, I could show you some 3 or 4 year follow-ups on six or seven patients with their renal functional patterns prior to diuresis, and then after diuresis following a single course of ACTH therapy and subsequently followed over a period of 3-4 years. In essence, these data show that regardless of the level of renal function at the time diuresis was initiated, if remission persisted, there was return of all renal functions to normal levels, where they have now remained for a period of 2 or 3 years

It is based upon this that we believe that ACTH or cortisone may be of distinct benefit in the management of children with nephrotic syndrome; that these drugs may not represent simple diuretic agents. We do not know what happens in diuresis from other causes. It is conceivable that any diuresis which is followed by remission could be productive of the same results. We have had an opportunity to observe two children who have had spontaneous remissions and followed them; one, for 3, and the other for only 2 years, with renal function studies, and both of these also have normal renal function.

MEMBER: Was their function low before they had diuresis?

DR. METCOFF: No.

MEMBER: Jack, you said you were perfectly sure that maintenance therapy has helped these children. How are you sure?

DR. METCOFF Well, I am sure of that on the basis of purely emotional and subjective grounds. (Laughter) I believe that currently there are no other criteria by which this can be determined, except by clinical experience and these are subjective grounds at best. On the same grounds, I am equally sure that there are some children who have not been benefited by maintenance therapy.

DR. LANGE· What do you call maintenance therapy?

DR. METCOFF· Well, what I call maintenance therapy, Dr. Lange, was recommended about three years ago at these meetings by you. (Laughter)

DR. LANGE: That is not good enough any more. I won't accept it.

DR. METCOFF: Well, in general, we give patients immediately following diuresis, cortisone, 300 to 400 mg. per square meter a day for 3 or 4 days out of each week.

Our longest period of observation in about 20 such patients is now in the neighborhood of 1 1/2 to 2 years

MEMBER: For how many successive courses did you give them?

DR. METCOFF: It has been about 1 to 1 1/2 years now in a few of these particular patients, others have not been followed for as long.

In conclusion, I should like to emphasize one thing about all evaluation of such data which seems to me very important and oftentimes neglected· Examples are 1) The determination of the selection of individuals who receive one type versus another type of therapy. For example, in our group, it is impossible for us to compare our statistics because patients oftentimes are given cortisone because we are afraid to give them ACTH. 2) The use of cortisone at home is dependent upon the cooperation of the parent and accessibility to the clinic. This is again a weighting factor. 3) The response of a child who has sustained remission following a single course of ACTH to maintenance with cortisone therapy cannot be compared with the response of a child who requires repeated courses of ACTH. Obviously, the second child either has more severe disease or other factors cause relapses which require repeated therapy.

One final point that should be re-emphasized -- it was made some time ago in these meetings -- is that many of us suspect our nephrotic population is changing, and has continuously undergone change A group of patients one sees in one year may not truly reflect the responses of a group of patients seen in another year. Furthermore, each year we are left with an increasing number of patients who have not responded to therapy The preparations with which we treat these children are also not above suspicion as they may be undergoing changes. So that the responses of a group this year oftentimes cannot be adequately compared with the response of a group of last year

I introduce these variables because I think they are important and must be kept in mind if data are to be properly evaluated.

MEMBER Jack, are the patients that you are giving maintenance therapy to, are many of them going through diuresis and becoming protein-free, or do they run all the directions of the gamut?

DR METCOFF: Every direction

We have quite a few patients as you can see now who are approaching a protein-free state of the urine. During this period of time we have encountered 21 patients who have received no steroid therapy whose urines are also protein-free following spontaneous diuresis.

MEMBER· Have you seen children reaccumulate while you have been giving them this course?

DR METCOFF· Yes.

MEMBER: Were they completely edema free when you started therapy?

DR. METCOFF: Yes.

DR. LANGE: We have seen children on 300 mg cortisone that have reaccumulated while they were on this weekly diet.

DR. METCOFF: Yes, we have two, and one of these patients had been protein-free for at least a year

DR. LANGE: But they were on it?

DR. METCOFF. She was on it faithfully, and had been edema-free for 15 months and protein-free for several months prior to the time she reaccumulated.

DR. FOX: Apropos of your comment about the selection of patients and so forth, I think it's extremely important to go back to a very old and fundamental principle of alternation of patients. I think we should consider that seriously.

DR BARNETT: This is quite right in principle, but it is awfully hard to do, as we talked about it before because you can't give some of the patients one thing and some of the others another.

DR. METCOFF: Let's assume for a moment that it were possible to set this up which I think it is I think this would be an ideal program to be centralized in the offices of the National Nephrosis Foundation. This also would be an ideal program for the Medical Advisory Board. Any decision, however, implies careful consideration of the moral problems involved.

Would you care to find out from the group whether they are all for such a program, if it should be set up, and if so, would they participate? This is the first thing before us.

CHAIRMAN RAPOPORT: Yes, we would participate. I have no reservation at all about participating in a program such as this.

DR. COOKE: If these were done properly, one of the series would be absolutely no therapy whatsoever That bothers me tremendously. No matter how dubious one is about the eventual benefit from ACTH, most people would agree that there are fewer markedly edematous nephrotics now than there were several years ago. How one justifies prolongation of morbidity by withholding all treatment, I do not know.

DR. BARNETT: You know, Milton, the details of this will obviously have to be planned by a committee, but to me the real basic question is whether these hormones are affecting the course of this disease or not - rather than, is this way of using them more effective than that way of using them. If this is the real problem, then the only real control is a group treated with the hormones, and a group not treated with the hormones.

MEMBER: Yes. Well, if you can induce diuresis with dextran?

DR. COOKE: I wouldn't be satisfied with that at all. I can remember the action of acacia which was a good diuretic but a very harmful one. I have the same suspicion regarding albumin. I don't think we know enough about dextran and, therefore, I would not want to use this as my control and compare this group with groups treated with ACTH or cortisone. I think that might be very misleading. I think it is "nothing" and "something," and the "nothing" bothers me a great deal.

CHAIRMAN RAPOPORT: We ought to get together and honestly present whatever material we have, and try to evaluate it. If necessary, turn it over to somebody who can treat it in terms of defining what all the categories are and then set up some program, feeling that while you can't have an acid test, you can have as close to one as possible.

DR BARNETT We may have more information than we think we have now, without a control series, that is if we really put all of our data together, as it is right now, and looked at it together, we might have some trend more than we think we have. I don't think I have any idea as to whether the use of the hormones has changed the course of the disease

CHAIRMAN RAPOPORT· Well, Henry, then as a starter would everybody be willing to gather all his cases back, let's say, for five years, or six years, and pool them?

DR METCOFF Could we agree on this one point? To give this consideration for several months instead of waiting for a year. Couldn't a few people get together and perhaps make some progress with a form, and with an evaluation of our existing material, so that perhaps by next year we would have some material in some kind of order?

MEMBER· Yes, I think we could do this I think it would be the rightful function of the medical advisory board of The National Nephrosis Foundation.

CHAIRMAN RAPOPORT: Dr Barnett, just as a starter, suppose I take you and Dr. Riley to expand yourselves into a committee from New York, so that phone calls aren't toll calls, and the rest of it can be carried on by correspondence.

DR. BARNETT. I think this would be reasonable since there are more of us in one locality there than any place else. Drs Riley, Kramer, Lange, Fox and myself since we can meet together easily, will take it upon ourselves to start it.

May I thank you, Milton, for myself and for this group for this meeting in its every aspect. I think these conferences have reached a high point with this meeting, and I think we all owe you a very great vote of thanks. (Applause)

CHAIRMAN RAPOPORT· The meeting is adjourned until next year.